FIELD ENHANCEMENT OF THE PARASITOID *Bracon hylobii* (Hymenoptera: Braconidae) TO CONTROL *Hylobius abietis* (Coleoptera: Curculionidae); THE LARGE PINE WEEVIL.

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ABSTRACT

Hylobius abietis (Coleoptera: Curculionidae), the large pine weevil is the most damaging pest of reforestation sites in Ireland. This study examined the possibility of manipulating populations of a species specific parasitoid, *Bracon hylobii* (Hymenoptera: Braconidae) to reduce weevil populations below damaging levels or to a level where they may be of use in an Integrated Pest Management system.

Levels of parasitism were studied in the field on recently felled Sitka spruce and Lodgepole pine sites in an attempt to measure 'natural' rates of parasitism. Highest rates of parasitism measured were 42% and 21% on Lodgepole pine and Sitka spruce sites respectively. This was very much lower than required for control.

Field studies of parasitoid emergence and laboratory trials indicated that two peaks of adult emergence occurred during the year. Synchronisation of peak emergence with the most vulnerable stage of the weevil is discussed. It may be that an ample portion of the *H. abietis* population survives to cause economic damage no matter how many parasitoids are on site due to overlapping of generations.

Attempts to enhance the populations of the parasitoid in the field failed due to migration of the flying adults. Reasons for this were investigated through field surveys of available plant food (nectar) and laboratory choice chamber experiments. It was concluded that the need for food and possibly, mutual interference resulted in a net migration of adults from the sites in which they emerged from the cocoons.

Experimentally it was demonstrated that the presence of food resulted in greater longevity and hence longer periods for searching and oviposition. The presence of flowering plants onsite may result in greater levels of parasitism. However, this is inconclusive.

The possibility of a hyperparasitoid being present was investigated using mtDNA techniques. No evidence was found to indicate the presence of a hyperparasitoid.

This study indicates that natural levels of parasitism in recently clearfelled Lodgepole pine and Sitka spruce forest sites is insufficient to control *Hylobius abietis*. The ability to enhance 'natural' populations by introducing laboratory reared populations failed because of the tendency of adult parasitoids to disperse. No hyperparasitoid is present.

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1 General Introduction

Due to Ireland's island status and relatively young forestry industry, forests on the island are regarded as being some of the healthiest in Europe (Anonymous 2002). This view is also recognised within the European Union Plant Health Directive 77/93/ECC, as Ireland is afforded protected zone status for 10 quarantine forest pests and diseases (Anonymous 2000). Forests in Ireland now account for approximately 10% of the land area, an increase of about 9% since the start of the 20th century. Much of this increase has occurred in the last 20 years due to attractive incentives available to farmers who afforest their land (Anonymous 2003). Many of these afforestation sites are largely free from attack by any serious pests or disease. The most serious and important pest and diseases of forestry, which occur in Ireland, are mainly a problem of re-afforestation sites. Of these, *Hylobius abietis* (the large pine weevil) is the most damaging insect pest of newly planted reforestation sites in Ireland (Ward 1993). The adult pine weevil causes damage to young plants, both conifer and broadleaf, on reforestation sites by feeding on the stems of the young plants. If the stem is completely girdled, due to feeding, the plant will die and will have to be replaced.

1.1 The Problem

Of course the underlying problem is *H. abietis*, a very well established pest with no possibility of eradication. Therefore the problem is one of control of damage to transplants and the solutions to how this control is achieved.

To date, prevention of damage to transplants rather than measures to reduce population levels of the pest has been the norm. This has and is being done by chemical means. Pesticides containing the active ingredients; lindane, permethrin and more recently Alpha-cypermethrin and cypermethrin have been used to control weevil damage. These chemicals are usually sprayed on the plants shortly after planting or the plants are dipped prior to planting. It would not be unusual for both treatments to occur. As regards spraying, there can be two applications per year for the first two years until such time as the plants are old enough and have a stem diameter which is less susceptible to total girdling.

For various reasons, discussed in detail below, achieving control of damage to transplants by chemical means because of environmental issues and the legality of their use, is becoming a major problem for forestry companies.

1.2 Why is control by pesticides a problem?

Commercial forestry is quite young in Ireland with most of the privately owned forests still in their first rotation. However, Coillte, a semi-state company responsible for the management and expansion of the public forest estate are involved in reforestation due to their inheritance of older age structured state forests. The company reforests approximately 7000ha per annum (Anonymous 2003), most if not all, of this would have to receive some sort of chemical protection against *H. abietis*.

One could argue that the use of pesticides or indeed other chemicals in forestry operations is very low when compared with mainstream agriculture. For instance Coillte, the largest landowner in the country (approximately 7% of the total land area or approximately 445,000ha) is responsible for only a fraction of the total pesticide used in plant protection in Ireland, applying less than 1% of the total (Anonymous 2007). However it has to be noted that pesticide use is concentrated on a relatively small area i.e. circa 7000ha.

Currently there is very little pesticide use in the remaining private plantations as the majority of these are still in their first rotation (Anonymous 2008). Therefore the amount of pesticide used by Coillte would be a figure very close to the total pesticide used in Irish plantation forestry.

In 2007 (latest figures available) Coillte reforested approximately 7157ha and used , on average, 0.102kg a.i /planted hectare, this would amount to a total of 730.1 kg of a.i being used in that year compared to 1793 kg of a.i in the year 2003. This decrease was partially due to the move from permethrin to alpha-cypermethrin (Anonymous 2005). At present Coillte uses 'Forester' (active ingredient: cypermethrin) to control pine weevil. Plants are either pre-treated in the nursery (dipped in 0.6% cypermethrin + Flexcoat), or sprayed following planting 0.2% cypermethrin. The adjuvant is Flexcoat, which sticks the chemical to the plant (thereby reducing the breakdown of

the product, and prolonging the period of efficacy). Where Flexcoat is used, there is generally no need for a top-up spray. Where a top-up spray is applied to dipped plants, this is generally not required until the year after planting (A. Dillon 2009, pers. comm., 31 Aug).

The use of chemical control in plant protection has a number of problems connected to it:

1. The location of many of these forests is of concern in relation to pesticide use. Traditionally in Ireland, mainly due to government policy, forest plantations were confined to poor or marginal agricultural land (O' Carroll 2004). Many of this afforestation in the mid 1900's took place on peat often in very sensitive water catchments. It is the possible pollution of important aquatic systems by runoff, spillage and drift of pesticides that drives efforts to reduce their usage.

2. If the system of clearfelling and replanting which is the main silvicultural system used in Ireland today is carried forward to the ever-maturing private estate then the problem of damage due to *H. abietis* will become a very important issue for private growers also. Should the use of chemical control of the pest continue in a similar fashion, as it is today, then the amount of chemical usage in forests will greatly increase.

3. The reliance on their use has disadvantages for both the environment and the users. Even with great care and adherence to all the safety measures when using these chemicals it is difficult to avoid exposure at some level. The most recently used Alpha-cypermethrin and cypermethrin based pesticides can cause skin irritations in humans (Anonymous 2006). Environmental consequences may include run-off into drains and streams, especially in the often characteristically high rainfall locations of many of the forests in Ireland (rainfall ranging from 1000mm to >2800mm, Met Eireann, 2012). Unintentional damage to beneficial organisms is also a concern as Alpha-cypermethrin and cypermethrin is highly toxic to bees and aquatic life (Anonymous 2006).

4. Many of the chemicals used in the past were banned or taken off the market for use in forestry. As one chemical became unavailable another was needed to replace it. Today the success of any forestry company is dependent on it being certified as sustainable. Sustainable forest management (SFM) is based on three pillars, economic, social and environmental and in Ireland is awarded by the Forest Stewardship Council (FSC). The FSC has a list of 'highly hazardous pesticides', Alpha-cypermethrin and cypermethrin are both included in this list. Any chemical on the list is prohibited for use on lands certified by the Forest Stewardship Council (FSC) unless a normal derogation or a temporary exceptional derogation for use has been formally approved by the FSC Board of Directors. Coillte has applied for a derogation to continue using cypermethrin, and are still awaiting a response. The UK has been successful in its application to continue to use both alpha-cypermethrin and cypermethrin (A. Dillon, pers. comm.). However the derogation lasts only 5 years after which alternatives must be sought. In an application for derogation a forestry company must outline how they are trying to find alternatives to using the banned chemical. These will most likely include efforts to promote integrated pest management (IPM) including the use of biological control where possible.

1.3 Possible solutions

The negative impacts of relying solely on chemical control for this and other forest pests and diseases, together with both Global and EU concerns related to the use of chemicals in forestry has lead to greater emphasis on integrated pest management (IPM).

The Irish forest service through the National Council for Forest Research and Development (COFORD) have in recent years funded projects such as the one described in this thesis to research potential ways in which chemical usage in Irish forestry can be reduced.

1.4 Why control Hylobius Abietis?

If left untreated *H. abietis* can cause up 95% -100% mortality of transplants (Ward 1988; Heritage *et al.* 1989) on sites with high population densities of the insect. The industry standard is currently 90% of original stocking, transplant survival in the first

4 years. Therefore, almost all clearfelled reforestation sites need to be protected against *H. abietis*. As mentioned previously, *H. abietis* kills transplants both coniferous and broadleaved by adult feeding on the phloem tissue of the transplants (King and Scott 1974). If the stem is completely girdled the plant will die (Wainhouse *et al.* 2007). Many transplants which are fed on are often not completely girdled but there may be sufficient damage to cause wind snap. Adult weevils can live for more than 4 years and stumps on clearfell sites can remain suitable for breeding for up to 3-4 years which means transplants are at highest risk of damage or mortality from *H. abietis* during the first 3 years after felling. The risk of mortality decreases after year 4-5 as the transplants increase in girth and the population of *H. abietis* declines (Heritage and Moore 2000; Evans *et al.* 2004). For these combined reasons *H. abietis* is the most important pest of reforestation sites in Europe (Heritage *et al.* 1989; Orlander and Nilsson 1999).

On average Coillte reforests approximately 7000 ha/annum. In the 3 year period from 2006 to 2008 Coillte's *Hylobius* control programme cost €6.82m, divided into €1.04m on pre-plant treatment and €5.78m on post plant spraying. During this period 72.4m plants were used in restocking of which 20% (14.7 million) were pre-treated plants. Depending on levels of potential damage the cost of weevil control can vary greatly from site to site, but where a site uses pre-treated plants and one top-up spray it costs approximately €350/ha (A. Dillon 2009, pers. comm., 2 Sept).

Both the cost and, more importantly, the detrimental effects on the environment of protecting transplants by chemical means has lead to an increased need for an integrated pest management approach to the problem, minimising the use of chemicals while promoting the use of alternative biological controls and silvicultural systems where possible.

1.4.1 *Hylobius abietis* History and life cycle

Hylobius abietis was thought to be relatively rare in England up to the early 1900's however it became widespread in both England and Ireland shortly thereafter. The spread in England is thought to have been in a north to south direction from Scotland. Its introduction to Ireland is mostly likely to have occurred through trade

with England or Scotland (Munro 1928). The gradual increase in *H. abietis* populations in Ireland most likely coincided with the onset of state afforestation programs which commenced in the early 1900's (Anonymous 2008).

Hylobius abietis is a member of the family *Curculionidae* which forms part of the super-family *Curculionoidea* of the order *Coleoptera.* The family *Curculionidae* contains many species that are important pests of both agricultural and horticultural crops and forest trees for example: Alfalfa weevil (*Hypera postica*), Clover root weevil (*Sitona lepidus),* Beech leaf miner (*Rhynchaenus fagi*) and The black vine beetle (*Otiorhynchus sulcatus*) to name but a few. However *H. abietis* is the only member of the family which is of any significance to Irish reforestation sites.

The adult *H. abietis* can range from 9.5mm to 14.5mm in length with an average length of about 12mm-13mm. Like other weevils *H. abietis* has a snout (rostrum) shaped head with a mouth and antenna located at the end of the rostrum. The antennae are elbowed and each femur has a notch and tooth like appendage on the inner or lower edge. Small groups of narrow long hairs are arranged in an echelon fashion on the elytra. These features can be used to identify *H. abietis* from other closely related weevils (Figure 1.1).

Figure 1.1. Adult H. abietis feeding on sapling[1]

[1] Image reproduced with permission from Wainhouse *et al*, 2007, (Images © Crown Copyright. Courtesy of Forest Research).

Hylobius abietis colour can be used as a relative guide to age. Immature weevils emerge from the stumps with a reddish brown colour and transform to a brown and then black colour as the chitin hardens. With the proper climatic conditions *H. abietis* can fly by extending the folded transparent wings located underneath the elytra. A saucer-like depression on the underside of the last segment of the adult male distinguishes it from the female (Anonymous 1960).

In a newly felled site the large area of cut stump surfaces emit volatiles which attract adult *H. abietis* from surrounding or nearby forests (Nordlander *et al.* 1986; Nordlander 1987; Zagatti *et al.* 1997; Leather *et al.* 1999). In Ireland most if not all of this migration is done by walking as temperatures are rarely high enough for a long enough period of sustained flight. However adult short flights on very hot days may slightly aid migration in Ireland (pers. ob). Flight and indeed swarms are a regular occurrence in mainland Europe. The inability to achieve sustained flight in Ireland does not greatly suppress *H. abietis* ability to travel however, as it is a very efficient walker travelling distances of up to 2km in a few days (Heritage and Moore 2000). Other methods of migration include transportation on logs and forest vehicles moving to and from forests and mills etc. Estimates of numbers of weevils invading new sites vary from author to author as does the distance travelled. However it would not be uncommon to see 3-5 weevils on transplants which would give a rough estimate of between 7500 and 10000 weevils per hectare (pers. ob). Other estimates of populations post spring time immigration have been put at 14000 adults per hectare (Nordlander *et al.* 2003). Nordlander's estimate is probably more accurate as not all weevils will be on plants at the same time. However estimates of between 65000 (Ward 1991) and 100000 (Dillon *et al.* 2006) weevils per ha on reforestation sites have been made. Total weevil numbers on a given site will vary over time and as the breeding and feeding resource dwindles. However it is possible that young weevils arriving and ovipositing in a site could remain for up to 3 years and so could give rise to as many as three generations of adults on a site over a period of time. It is generally accepted that weevils, especially where they use flight can travel up to 10km. Studies by the Forestry Commission in Scotland have suggested that individual weevils are capable of migrating 1km in approximately one month to a new clearfell site. (Moore *et al.* 1998). The ability to cross roads, ditches, drains and

other obstacles was confirmed by (Munro 1928). Adult weevils migrate to new sites usually during the spring to autumn period when temperatures are high. The actions of the female weevil once she arrives to a new site will differ in relation to the timing of her arrival and her reproductive state. If she has mature fertilised eggs and arrives in early spring she will usually feed and oviposit quite quickly. However if the female is newly emerged she will have to feed to become sexually mature, then mate and oviposit eggs. On the other hand if the immigrating female arrives in the late summer and has already laid a large amount of eggs previously she may overwinter on the site and not oviposit until the following year. Following the winter she may have to mate again and or mature more eggs. Overwintering before oviposition may also occur in late emerging females. These females will oviposit in the spring of the following year, approximately two weeks from the end of the hibernation. It is a requirement of both sexes to have overwintered at least once in their development to become sexually mature

The females lay their eggs in stumps, logs or branches of most conifer species in contact with the ground. Some evidence has shown that the eggs may also be laid in the soil close to the stump, log or branch (Nordlander *et al.* 1997). Even though *H. abietis* will feed on broadleaf trees, they are not used as breeding sites (King and Scott 1974). To prepare an oviposition site the female chews out a notch in the bark usually below the soil surface into which she lays a single off-white oval shaped egg. However any notch or opening, created by other factors, which are suitable can be utilised by the female. A female may lay several eggs in close proximity to each other or space them throughout an area in a given stump suitable for oviposition. First instar larvae can travel up to 50mm in the soil to suitable feeding material. As weevils are immigrating and emerging at different times throughout the season the egg laying period reflects this with oviposition continuing from early summer to early autumn. Peaks occur during May and June. Novak, (1965) as cited in Henry (1995) found that female *Hylobius* laid an average of 118 eggs per season. Research carried out in the University of Ulster found that the mean number of eggs laid by female *Hylobius*, over a period of 17 days, after feeding on four different substrates, was 0.1-3.7 eggs/day (Thorpe and Day 2008). Temperature affects the metabolic rate of insects (poikilothermic) so therefore their growth, activity and development will

increase with an increase in temperature (Martin *et al.* 1999). Eidmann, (1974) as cited in Henry (1995), found that the development time of the *Hylobius* eggs ranged from approximately 34 days at 7°C to 6 days at 25°C. Total development from egg to adult in natural conditions ranges from 12-36 months (Leather *et al.* 1999).

The larvae develop through 5 instars eventually reaching a size of approximately 25-30mm and weigh over 800mg (King and Scott 1974; Henry 1995). The size of the larvae can be used to determine the development stage of the larvae; however care must be taken as there can be an overlap in size between instars (Wainhouse *et al.* 2007). The larvae are slightly C shaped, with a quite large flattened brownish-red head capsule (Figure 1.2). The body is quite soft to the touch and can withstand a fair deal of applied pressure. The head capsule is almost equal in length to the first segment of the body. As the larva develops from one instar to the next the head capsule splits and reveals a pale whitish coloured head capsule beneath. This quickly hardens and regains the brownish-red colour.

Figure 1.2. Development stages of *H. abietis* larvae[2]

The larvae feed on the cambial tissues between the outer bark and inner wood. The feeding behaviour of the larvae is influenced by the substrate in which they are feeding. This in turn is usually as a result of the characteristics of the tree species from which the feeding substrate is derived. Weevil larvae feed in solitary galleries, in tunnels which get progressively wider as the larvae grow and are packed with frass

[2] Image reproduced with permission from Wainhouse *et al*, 2007, (Images © Crown Copyright. Courtesy of Forest Research).

as the larvae progress. The location of the tunnels can differ with regard to the thickness of the bark. In pine, which has quite a thick bark the weevil larvae tend to feed entirely within the bark often creating vertical ventilation tunnels almost to the surface of the bark. If the bark is thin in a species like spruce the feed tunnel may encompass part of the inner wood as well as the bark with only a very thin layer of outer bark concealing the tunnel. This fact may have implications as regards the availability of the larvae to predators and the ease of which it can be parasitised for example, the parasitoid may find it physically easier to probe thinner bark.

Larval development is greatly influenced by climatic conditions and in particular the surrounding soil temperature of the breeding site/stump. In laboratory conditions at room temperature eggs have developed to fully grown larvae in less than 2 months (pers. obs). In the field it can take an egg a year to develop into a fully grown larva, again however this is influenced by time of egg laying. An egg laid in early spring receives the full benefit of high summer temperature and therefore can develop into a fully grown larva ready for pupation by the autumn of the same year. In this case overwintering occurs as a pupa with development to adult continuing in the spring/early summer of the following year. If egg laying occurs late in the season the onset of winter and lower temperature delays further development until the spring of the following year. In this situation the larva will overwinter and will spend a large part of the following season continuing larval development and pupation finally emerging as an adult in the summer. If conditions for development are poor throughout the season there maybe two overwintering periods before adult emergence in the third season. An illustration depicting the life cycle of *Hylobius abietis* is shown in Figure 1.3.

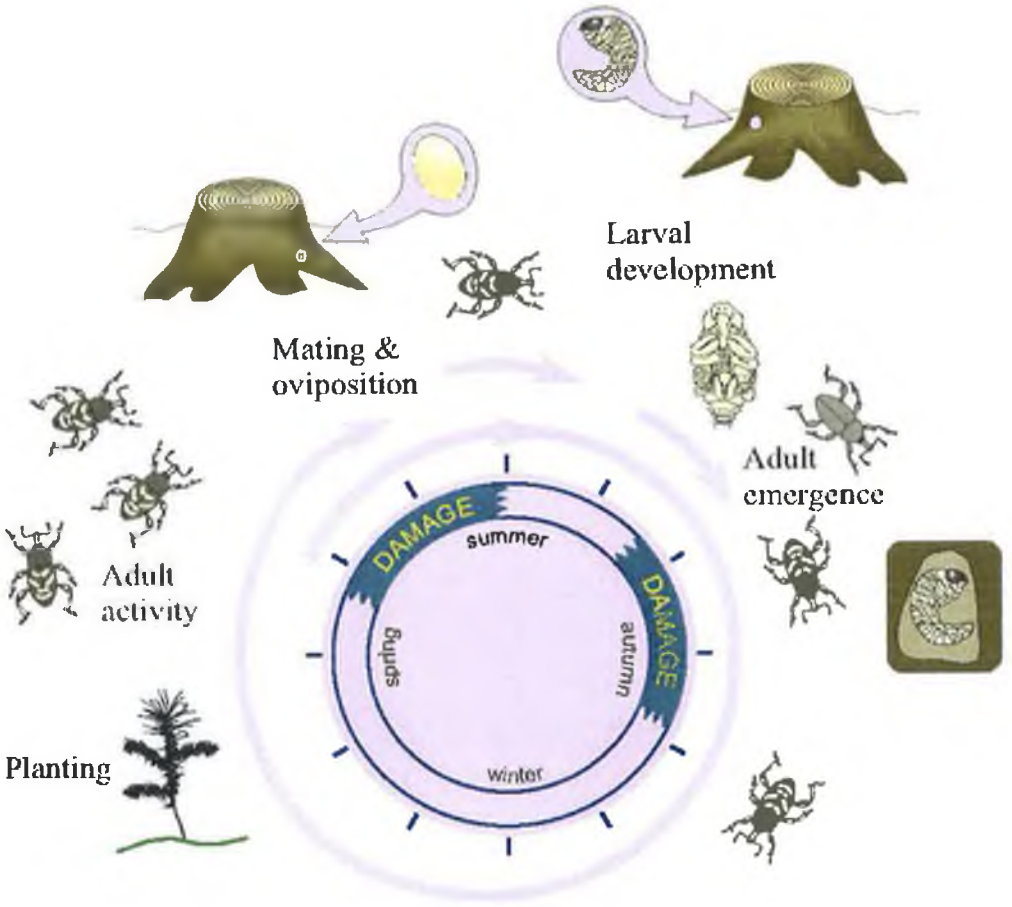

Figure 1.3. ***Hylobius abietis*** **Life Cycle**[3]

The location and construction of pupal chambers is usually influenced by the thickness of the bark in which the larva was feeding. As mentioned previously if the bark is thick and feeding tunnels are contained totally within it, then it is likely that the larva will form the pupation chamber within the bark. This is common in pine and often when recording larvae in stumps one would have to physically break the bark into pieces after stripping it off to ensure all larvae are recorded. Fortunately however the chambers constructed in the bark seem to create weaknesses along which the bark often tends to split. If the bark is shallow the chamber may be partially constructed in the wood and the bark, this gives rise to the occurrence of shallow oval shaped depressions in the wood occupied by the pupa, which is visible

[3] Image reproduced with permission from Forestry Commission UK, (Images © Crown Copyright. Courtesy of Forest Research).

when the bark is removed. If the bark is very thin, or if there has been a high population of larvae in the stump, and all of the feeding material is consumed, the larva will tend to burrow into the stem and form a chamber running vertically just under the surface of the wood. The only visible sign of such chambers is a circular hole in the wood of the stem often packed with the excavated woody fibres. The location of the chamber and thus its level of concealment will influence the availability of larvae and the ease with which a parasitoid can attack the larva, within the chamber (Henry 1995; pers. ob).

If the larvae pupate and emerge as adults in the same season, the time from initiation of pupation to emergence of the adults is approximately 4-5 weeks. This includes a period of 14 days pupation followed by a resting period of between 13-20 days in the chamber before emergence (Novak 1965. cited in Henry 1995). The adults exit the chambers by chewing through the bark. If overwintering occurred in the larval stage with further larval development required in the following season then this pupation and resting phase will occur during July-August of the second season resulting in emergence and large amounts of feeding damage in August of the second season. When the overwintering occurs in the adult stage then emergence and damage will occur in the spring of the second season or third season if development was very slow. This results in peaks of feeding damage in the spring and autumn (Moore 2004). As it is more common for *H. abietis* to overwinter as larvae than adults the feeding peak in the spring is often more as a result of migrating weevils combined with the newly emerging adults. However, damage in the autumn can be largely attributed to the large numbers of newly emerging adults. Emergence and feeding can occur throughout the season at low levels due mainly to overlapping of generations and varying development times depending on site and climatic conditions (Heritage *et al.* 1989; Leather *et al.* 1999; Heritage and Moore 2000; Wainhouse *et al.* 2007).

After a period of feeding the females become sexually mature and mate thus beginning the cycle again. If the area contains suitable breeding sites the female will usually remain and lay eggs again if suitable breeding sites are scarce she will migrate to a new breeding site. Depending on climatic factors and tree species, sites

can remain viable for breeding for up to 3-4 or even 5 years. However in the west of Ireland and similarly in the west of Scotland where the climate is mild and wet, deterioration of stumps is often much quicker and along with the presence of stump degrading fungi such as *Armillaria sp.* and *Heterobasidion annosum* and often high water tables stumps can be rendered unsuitable as breeding sites in the second or third season (Munro 1929; pers. ob)

1.5 Non chemical Control of *Hylobius abietis*

Munro (1914) indicates that Ratzeburg and Nordlinger carried out some research on parasitoids of *H. abietis* in the early 19th century. Munro himself seems to be one of the earliest researchers into the non-chemical control of *H. abietis* in the UK. He describes work he carried out on a braconid parasitoid of *H. abietis* which he was happy to agree was the same as a parasitoid Ratzeburg and Nordlinger had identified and which Ratzeburg named *Bracon hylobii.*

These seem to be some of the earliest accounts of non-chemical bio-control of *H. abietis* in Europe and particularly the UK. Since then and up until the present day a great deal of research has been carried out concerning the control of *H. abietis* by non chemical means.

This research could be broadly divided into 3 categories

- Physical barriers, Traps and Antifeedants
- Habitat/ breeding site manipulation
- Biological control

1.5.1 Physical barriers, Traps and Antifeedants

Physical barriers are, as their names suggest, appliances which physically prevent the pest from feeding on the crop/plant under protection. The barrier can be a shield with or without a collar placed around the stem or a coating of some type, applied to the stem. In relation to *H. abietis* different designs have been tested and products such as Bugstop, Hylostop and Snapguard have been developed. Bugstop is basically a mineral wax coating applied to the stem of a new transplant while Hylostop is a shield without a collar, made from PVC coated paper with an external coating of polytetrafluoroethyhene that is placed around the seedling at planting. The polytetrafluoroethyhene coating is made up of small particles, which cause the pine

weevil to slip when it tries to climb up the shield to feed on the plant. Snapguard is a narrow tube of polypropylene which snaps shut around the stem of the plant it has a collar on the top to prevent the weevil from climbing over it (Eidmann *et al.* 1996; Petersson and Orlander 2003; Petersson *et al.* 2004). There have been various other coatings and shield tested but all are similar in principle to the three mentioned above.

Research has been carried out to compare the mortality rates of new transplants/seedlings protected by feeding barriers and those protected by insecticide. Petersson *et al.* (2004) compared the success of different feeding barriers versus insecticide (permethrin) in protecting new seedlings on clearfell sites over a 6 year period in Southern Sweden. They found that after 3 years the coating and shield barriers without a collar were the least effective (47% and 59% mortality of seedlings respectively) when compared to the insecticide (13%). The shield barriers with the collar however offer the same level of protection (26%) over the 3 years as the insecticide. All treatments showed better survival rates than the control (87%) after 3 years. In comparison to this research Eidmann *et al.* (1996) found that polytetrafluoroethylene coated shield barriers decrease the mortality of seedlings caused by *H. abietis* feeding in one year by 93% and by a mean of 84% over two years.

Informal trials on the use of feeding barriers (mainly shields) in Ireland have been carried out by interested Coillte foresters. Anecdotal evidence would suggest that they have not been as successful as in Scandinavia. In Ireland many reforestation sites tend to be on peat, this substrate is not ideal for the use of shields as when the peat dries and cracks in the summer the shields often move and allow the pine weevil access underneath (P. Blighe 2005, pers. comm., 7 July).

Trapping weevils using billets traps (small freshly cut logs used to attracted weevils, which are then collected and destroyed) and pit traps with the same idea were used in attempts to control weevil populations on clearfell sites in England and Scotland in the early 20th century (Munro 1929). Although these methods were largely unsuccessful at reducing the population of *H. abietis* below an acceptable level

Munro argues that it was the timing of trapping that was a major cause of the lack of success. He suggested trapping weevils in the crop 2 to 3 years prior to it being felled rather than after it had been felled. The reasoning behind this was that populations pre-felling were low and so may be reduced to a more acceptable level. Rather than trying to reduce high populations post felling. He reports having some successes with this method and suggests it could be effective and less costly than other methods used at the time (Munro 1929). However this method did not receive much more attention and is rarely considered an option in reforestation sites today.

Antifeedants for *H. abietis* have mainly been tested in laboratory experiments and limited field trials. They include the use of neem oil extracts, repellent/protection coatings and biofungal treatments. All have the purpose of repelling and or inhibiting the feeding of the adult weevils. Laboratory and field trials carried out on neem oil have shown it to have a significant antifeeding effect. Treated trees remained unaffected by weevil feeding while all untreated control trees died (Thacker *et al.* 2003). In laboratory experiments *H. abietis* showed a significant preference for untreated seedlings compared to those treated with a protective wax coating namely CereNAt E30. However in the same study seedlings treated with a biofungicide containing spores of *Phlebia gigantea* (Fr.:Fr) Donk, showed no significant difference in attraction to *H. abietis* when compared to untreated seedlings (Watson 1999). Research testing a range of host and non-host woody plants for antifeedant qualities found that *Pinus* was the most attractive resulting in the highest feeding area while *Ilex* had only small areas of nibbling on the outer bark. All the species tested had feeding to some extent in the outer bark, however in some broadleaved species *H. abietis* stopped feeding when it reached the inner bark, suggesting that some antifeedant compounds are located in this area (Mansson and Schlyter 2004).

Plants themselves have a defence system which involves the development and storage of compounds during periods of normal growth. When a plant is attacked these readymade compounds act as a means of repelling or even killing the attacking herbivore (Gatehouse 2002). Other studies have concluded that volatiles emitted by herbivore damaged plants can attract parasitoids; this is a form of indirect plant

defence strategies (Geervliet *et al.* 1994; Turlings *et al.* 1995; Dicke and van Loon 2000).

1.5.2 Habitat/ breeding site manipulation

Hylobius abietis breeds in stumps of cut or fallen conifer trees (Eidmann 1974). The larvae themselves cause no economic damage as they feed on the cambium under the bark of these cut stumps. It is only adults that have the potential to kill newly planted trees by girdling the stem when feeding. All commercial control of *H. abietis* in recent times has focused on the adult stage of *H. abietis*, mainly through the application of pesticides. However the destruction or manipulation of *H. abietis* breeding sites, namely conifer stumps in an attempt to reduce the availability or quality of the breeding sites has gained momentum as a method of control.

The ability of saprotrophic stump colonising fungi (stump-degrading fungus) to both speedup the decay of the breeding site and also deter weevils from laying eggs in the infected stump has been investigated. Results of the numbers of weevil larvae present in inoculated stumps 12 months post felling and application of the fungus indicated that the treatment had no effect on weevil numbers. However, indications are that the fungus actively degraded the stumps and so could have a positive effect over a longer time. Results also indicated that some fungi may facilitate *B. hylobii* in accessing weevil larvae (Dillon *et al.* 2008).

Manipulating sites with a view to reducing *H. abietis* damage has concentrated mainly on silvicultural operations. These include burning, mounding, scarification, a change in silvicultural system or site management. The methods have shown varying degrees of damage reduction and in some cases have increased damage. Often a combination of these methods with reduced chemical application has proven to be the most successful (Heritage and Moore 2000). Any treatment that exposes mineral soil around the plant will reduce the damage caused by *H. abietis*. (Heritage and Moore 2000; Heiskanen and Viiri 2005; Petersson *et al.* 2005; Petersson *et al.* 2006). Burning, Mounding and scarification are common practices which remove or disturb the top layer of vegetation and humus to expose the underlying mineral soil.

However, while burning will expose mineral soil, it has also been shown to cause an increase, both in the level of severe feeding and mortality of pine seedlings on clear cuts when compared to unburned clearcuts, this increase was negated when there was retention of mature live trees on the burned site (Pitkanen *et al.* 2005; Von Hofsten and Weslien 2005; Pitkänen *et al.* 2008). When planting on a clearfell site, which has been burnt, a fallow period of at least 2 years is required to reduce damage caused by *H. abietis*. (Von Hofsten and Weslien 2005). Obviously burning will have the effect of removing alternative food sources of *H. abietis* from the habitat and therefore may have the result of concentrating feeding on newly planted sites.

Mounding is a mechanical site preparation, which involves the inverting of a patch of soil approximately 45 -60cm wide and 15-20cm high to form a mound into which a new seedling can be planted. This type of site preparation has been shown to decrease the rate of feeding by *H. abietis*. (Heiskanen and Viiri 2005; Nordlander *et al.* 2005; Petersson *et al.* 2006). Vegetation around a plant can act as a source of shelter in which *H. abietis* can safely feed. Planting in a vegetation free patch such as that achieved by mounding can reduce feeding damage on the plant (Petersson *et al.* 2006). The actual slope of the mounds themselves may also impede access of *H. abietis* to the plant (Nordlander *et al.* 2005).

Scarification involves the disturbance of the top 10-20 cm of soil either by manual or mechanical means. This removes competing vegetation and exposes the mineral soil. The main aim of scarification is to provide a new transplant with a competition free microhabitat in which to have good early development. However as with mounding scarification also has a positive effect on the reduction of damage caused by *H. abietis* (Lof 2000; Petersson and Orlander 2003). This again is probably due to the decrease in shelter/cover available for *H. abietis* and the increased growth rate of the transplants.

In order to control a pest one has two choices, kill the pest or limit its breeding and feeding resources. In the past the burning, burying and destruction of conifer stumps has been carried out on small areas. In theory it could be an effective method in the reduction of weevil numbers but is often too costly and impractical. One of the main reasons why these methods are not successful is that it is almost impossible to totally

destroy all the above and below ground parts of the stump. The fact that female weevils require only a relatively small portion of bark/stem even less than 10mm in diameter, in contact with the ground to lay and allow its eggs to develop (Heritage and Moore 2000) means that numbers will usually remain damaging even after such treatments.

1.5.3 Silvicultural systems

The clearcuting system, which prevails in Ireland, provides the ideal breeding feeding habitat for *H. abietis* (Wainhouse *et al.* 2001; Evans *et al.* 2004). Research in Finland suggests that *Hylobius* populations could be 10-24 times greater in clearfells compared to undisturbed forests (Pitkänen *et al.* 2008). In Ireland clearcutting involves the clearing of areas up to 25ha which are predominantly coniferous trees approximately 35-40yrs old, followed by the subsequent planting of 1-2 year old transplants. Clearcutting leaves large numbers of freshly cut conifer stumps which are the ideal breeding site for *H. abietis*. *Hylobius abietis* is attracted to the site by volatiles emitted from the cut stumps (Nordlander 1991; Lindelow *et al.* 1993). Females oviposit eggs on or near the roots of the recently cut stumps. If the site is replanted the new transplants offer a perfect food source to the immigrating adults. When the next generation emerge from the stumps there is an abundant food source in close proximity. This coupled with the fact that newly felled clearcuts, especially those on nutritionally poor peat sites often tend to be devoid of any other woody vegetation which can reduce the damage pressure on the transplants (Heritage and Moore 2000). Apart from brash the transplants offer the main source of food on such sites. This gives rise to often severe damage and high mortality rates of transplants.

Alternative silvicultural systems which have been shown to reduce the damage caused to new transplants by *H. abietis* are forms of shelterwood or selection systems. In a Shelterwood system mature trees are retained on site at the time of felling. Reasons why this system reduces damage are not fully understood but may include the fact that the fine branches and twigs of the mature trees are used as a food source by adult *H. abietis* (Heritage and Moore 2000; Petersson and Orlander 2003), the lower stems and roots could also be a source of food (Orlander *et al.* 2001). The fact that adults are feeding on the mature trees reduces the damage by feeding caused

to the transplants. As mentioned previously planting into scarified patches can reduce feeding damage by *H. abietis*, at least until the patches are re-colonised by vegetation, by reducing shelter and cover for the adults. Providing shelter trees can slow down the rate of re-colonisation in scarified patches and thus prolong the beneficial effects of the cultivation (Petersson and Orlander 2003).

Selection thinning practiced in Germany and other mainland European countries involves the removal of single trees or very small groups of trees across a range of diameter classes. Damage by *H. abietis* when this system is used is quite low. This again is largely due to the fact that there are other sources of food available in the standing mature trees. Pitkanen (2008) found that on clearfells with groups of retained trees, that walking and flying hylobius were attracted to the retained trees. The total amount of volatiles released from cut stumps in a selection system would be lower than that released in a clearfell thus less immigration of weevils would be expected. However in the Irish situation the economics of selection systems on relatively low value timber are questionable. The use of a clearcut system and its implications as regards to *H. abietis* in areas where the weevil is a problem needs to be reassessed.

1.5.4 Biological control

Biological control is defined as "actions of parasites, predators and pathogens in maintaining another organism's density at a lower average than would occur in their absence" (DeBach 1964). Ancient cave paintings in China suggest that bio-control in the form of the use of ducks to eat crop pests was used from the beginnings of agriculture. Later documented efforts occurred in Mauritius and Burma in the latter half of the 1700's. However the birth of classical biological control could be traced to California where ladybirds were used to control a citrus scale. These and subsequent efforts were largely driven by the lack of a cheap effective insecticide. Examples of successful bio-control attempts can be found up until 1935. The development of DDT around this time more or less quelled the interest in bio-control. DDT offered a cheap and effective control for many pests for many years. It was not until the negative effects of the extensive use of the chemical, such as the harm and death caused to other beneficial insects, bird and aquatic life was realised that the interest in bio-control rose again towards the 1970's (van Emden 1989).

Since that time research into bio-control has grown considerably and many very successful programs have been implemented. An estimated 16-34% of bio-control projects are successful (Hoy 1988).

As with insecticides bio-control has both advantages and disadvantages, which include:

- Compared with many insecticides biological control agents are more specific to the pest being controlled whereas insecticides usually will harm a much broader range of species outside the target pest.
- As many bio-control agents are very mobile organisms such as parasitic wasps or ladybirds, they can be released in an area needing control in which they search out and kill the pest. An insecticide must be directly applied to the crop requiring protection or the pest being controlled.
- Obviously the introduced bio-control has the ability to reproduce and therefore may only require one release. Depending on the persistence of a given insecticide it may have to be applied more than once in a season and usually every season thereafter.
- Pest have been very adaptable to building up resistance to chemicals used as controls however it is very difficult or impossible for a pest to develop resistance to another organism being used as a control.
- Insecticides can offer a high level of certainty regarding control whereas bio-controls can be somewhat hit and miss as a result of other factors such as site and climatic conditions.

To increase the chances of a successful bio-control program it is most important that the chosen control agent has the characteristics which will enable it to be an effective control. Analysis of the success or failure of a bio-control programme is often concentrated on the bio-control agent, however (Lane *et al.* 1999) suggest that growth rates, periods of susceptibility to attack and refuge characteristics of the host need to be focused on to a greater extent in any such analysis.

In any given situation a certain percentage of the target pest will be more easily accessible to the control agent than others. The bio-control agent will attempt to spend the least amount of energy in host searching as possible and therefore will

attack the more easily available hosts first. It is the ability or tendency to search out those hosts which are not readily available (concealed) before deciding to leave a site that makes a bio-control agent more effective.

As mentioned one of the main disadvantages to using insecticides is the fact that they do not distinguish between target and non-target species. This problem can be overcome by the use of a bio-control agent which is specific to the target pest. However not all bio-controls exhibit this characteristic and so it is important to select an agent which is specific to the pest. On the other hand if a bio-control agent is highly specific to one pest it leaves itself vulnerable to short term lows in the pest population density. If the control agent cannot sustain its numbers by parasitising alternative hosts while the pest numbers are low it risks local extinction itself.

It would be fair to say that the greater the numbers of a bio-control agent on a given control area the greater the increase in the rate of control will be. Thus the greater an agent's longevity and fecundity coupled with multivoltinism with a short development period per year the more successful one would expect it to be. This characteristic is even more important in a bio-control agent when the target pest also has many generations per year or overlapping of generations on a given site over a course of several years.

If a target pest can develop in low temperatures it is important that the chosen bio-control can also survive and develop at the same temperature. If this is not the case the susceptible development stage of the pest maybe missed by a control agent which needs a higher temperature for activity and development.

Finally if the chosen agent of control needs to be mass reared it is very important that this can be done with ease, cheaply and quickly. In some cases it is the difficulty of rearing hosts that can slow down or make a rearing program unfeasible. A control agent which can be reared on an easily cultured alternative host or on an artificial diet would be preferable.

In summary an effective bio-control agent should be host specific, have a high host searching ability, high fecundity and longevity. It should be adaptable to a range of climatic conditions and should be easily reared in a laboratory situation.

1.6 Methods of biological control

Once a bio-control agent is chosen it is then necessary to establish the way in which it will be used. There are three general methods for biological control which include: Introductions, Augmentation and Conservation. However there is crossover between the methods as for example the addition of extra non-host food on a site can be used as part of an augmentation and/or a conservation approach

1.6.1 Introductions

Introductions of foreign bio-controls are usually as a result of a previous introduction of an exotic pest into a region. The new exotic pest may not have been of economic importance in its home range but without the presence of it natural enemies, thrives and becomes a pest in the region of introduction. In this case it is often most suitable to search the home range for natural enemies which may be introduced to control the pest. However, it is often necessary to import, and trial a number of the pest's natural enemies as the climatic conditions, and/or other environmental factors, of the new region may differ and lead to an unsuccessful control program. Also it is possible that if the pest is present below an economic threshold in its region of origin that both it and it's bio-control agent are present at low population densities thus the most effective control agent may be hard to locate or overlooked (Ivie and Knausenberger 1979).

1.6.2 Augmentation

Augmentation in the form of mass releases is a method where the numbers of an existing bio-control agent are increased by the addition of high numbers of laboratory reared individuals of the same species. This is usually done by periodic releases of the control agent either through inoculative or inundative means (Cohen *et al.* 1999). Augmentation is often done in situations where conditions have favoured the development of the pest to a greater extent than the bio-control agent. A monoculture clearcut forestry system would be a case in point, where for instance the

system favours the pest by supplying numerous breeding sites and food whereas there is for example a lack of food in the form of floral nectar available for non host feeding adult parasitoids. In cases such as these it could be beneficial to augment the existing population with high numbers of laboratory reared individuals. The timing of such mass releases is critical and in the case of a larval parasitoid should be done early in the season, especially if there is a natural lag in the development of the bio-control compared to the host.

Augmentation can take two forms namely inoculative or inundative. Inoculative augmentation usually entails the release of relatively small numbers of the control agent in the early stages of the pest development or infestation. The agent should establish quickly and along with the next generation should decrease the population of the pest. Inundative releases on the other hand relies more on the release of huge numbers of the adult bio-control maybe several times in a year or over a number of years with any decrease in pest population being attributed directly to the released adults rather than their offspring. In an experiment involving the augmentation of two species of parasitoids against citrus blackfly in Texas, the two species were successfully re-established to a stable host-natural enemy relationship through the release of over 100,000 parasitoids in grapefruit and orange groves during a period from January 1992 to February 1993 (Meagher and French 2004).
As with introductions the control must be suitable for quick inexpensive mass rearing in laboratory conditions (Nordlund 1998).

1.6.3 Conservation

Conservation is concerned with the protection of a bio-control agent as regards trying to prevent any large reduction in its numbers and providing or enhancing elements in the control areas which would increase the control agents activity and survival. This method is usually used in relation to native control agents. Conservation methods include the use of selective insecticides which will minimise the effect on the control agent. Also the timing of insecticide application to coincide with periods of inactivity of the control agents can be beneficial. Adult parasitoids which do not host feed will have to search for food in the form of floral nectar or perhaps honeydew. The addition of such food sources to a site can have a conservation benefit as the adult

parasitoids can spend more time searching for hosts than food without the risk of starvation or forced dispersal (Takasu and Lewis 1995; Stapel *et al.* 1997; Lewis *et al.* 1998) If a bio-control agent reduces the number of a particular host to a level where it becomes difficult for it to find new hosts, one of two things will happen, the agent population will either collapse or if will be forced to move to new sites in search of new hosts. If however there were alternative non-pest hosts available on site in times of low pest numbers the control agent would be able to sustain a population on site, ready for periods of high pest numbers in the future (De Clercq 2002).

1.6.4 Factors affecting biological control methods

Introductions are often limited by the ability to economically produce large numbers of the control agent (Hoy 1988; Ulrichs and Mewis 2004). This has lead to research into artificial diets, as often it is the ability to produce the host easily that is a critical factor. However artificial diets for entomophagous insects remains unsuccessful and thus large numbers of predators are reared on natural diets (Cohen *et al.* 1999)

The mass rearing of parasitoids for augmentations or inductions often encounters the problem that more males than females are produced therefore increasing the costs per female produced. (Heinz 1998) in a study on the effect of host size on sex ratio in the parasitic wasp *Catolaccus grandis* (Hymenoptea: Pteromalidae) found that when the female perceived the host to be large (in relation to previously encountered hosts) she laid female eggs while laying males on the smaller hosts. The female larvae also developed quicker and to a greater size than the males. Studies on the parasitic wasps, *Metaphycus flavus* (Howard) and *M. stanleyi* Compere (Hymenoptera: Encyrtidae), biological control agents of citrus-infesting soft scales also concluded that the size of the host was a major factor of importance in trying to mass rear the wasps as both the number of females and the size of the clutch increased in respect to the host size (Bernal *et al.* 1999).

The coincidence of large numbers of the adult parasitoid with the vulnerable stage of the host is vital for successful bio-control. However if an overwintering population of a parasitoid develops slower in early spring than the susceptible stage of the host

then the host may escape attack. Thus, varying early spring temperatures can influence the development synchrony of the host and bio-control agent and therefore the rate of increase of the agent's population and success in control (Van Nouhuys and Lei 2004).

Research on the braconid wasp *Cotesia melanoscela* (Ratzeburg) found that knowing the time taken for adults to emerge from mass reared overwintered cocoons after they are transferred from cold to warm temperatures in the laboratory can greatly improve the synchrony of the host and bio-control as the adults can be transferred to the field at the right time (Webb *et al.* 1997).

Adult parasitoids which feed on sugars use carbohydrates as their main energy source, these sugars are taken from both floral and extra floral nectaries as well as aphid honeydew (Jervis *et al.* 1993). As many parasitoids are used in modern monoculture systems in both forestry and agriculture their target areas are often devoid of much biodiversity as regards flowering plants (Berndt *et al.* 2002; Wackers 2004). The addition, or enhancement, of flowering plants in a target area can increase the rate of parasitism by nectar feeding bio-control agents. This can be as a result of the agent not having to leave the target area to search for food and thus spending more time in the locality (Lewis *et al.* 1998; Faria 2005) and also the ability to feed frequently will increase adult longevity and fecundity and therefore parasitism rates (Cortesero *et al.* 2000; Manojlovic *et al.* 2001; Wackers 2001; Wratten *et al.* 2002; English-Loeb *et al.* 2003; Jacob and Evans 2004; Tenhumberg *et al.* 2006; Lee and Heimpel 2007) .Research on the parasitoid *Cotesia rubecula* found that it had to feed at least once per day to avoid starvation (Siekmann *et al.* 2001). In a separate study it was also found that only well fed *Cotesia rubecula* adults searched for hosts before food while host and food resources seemed to be equally attractive to unfed or hungry adult females (Siekmann *et al.* 2004). The speed at which a wasp uses its stores of body sugar will influence its survival time in the absence of a sugar source and could be an adaptation to the relative biodiversity of its main habitats i.e. slower use of body sugars in habitats with poor food resources (Vattala *et al.* 2005).

Research has found that by providing non-host food in the form of floral nectar from buckwheat and honey increased both the longevity and fecundity of two parasitoids, namely *Diadegma semiclausum* (Helen) which parasitises the diamondback moth and *Microctonus hyperodae* Loan which attacks the Argentine stem weevil (Wratten *et al.* 2003). Many other studies have shown the beneficial effects of providing food sources for adult parasitoids in relation to increases in longevity, and fecundity (Jervis and Kidd 1986; Eijs *et al.* 1998; Lee *et al.* 2006; Rose *et al.* 2006; Hogervorst *et al.* 2007; Irvin *et al.* 2007; Heping *et al.* 2008; Lee and Heimpel 2008; Witting-Bissinger *et al.* 2008).

The flowers used in habitat enhancement should be selected carefully as not all flowers are attractive to all parasitoids and some are even repellent. In addition, those that are attractive may not have accessible nectar. Therefore it is vital to test a range of flowers for a given parasitoid to optimise both attractiveness and accessibility (Wackers 2004). The synchrony of the flowering period of the plant with the period of release is of obvious importance also (Landis *et al.* 2000).

Even though some adult parasitoids and other nectar feeding insects may show an innate preference to a particular flower or flower colour (Wäckers 1994; Stapel *et al.* 1997), in cases where that flower may not be available they can also learn to associate different flowers and colours with a nectar reward (Weiss 1997; Oliai and King 2000), thus the abundance of flower on a site may be more important than the presence of any one flower species. A study on 250 species of parasitic wasps from 15 different families found that 32 of the 52 flowering species in the study were searched, and/or fed on by the wasps, however a point of note was that males and females of the same species often showed a preference for different species of plants (Jervis *et al.* 1993).

The non-target effects of mass released parasitoids is of great importance. If a released natural enemy attacks and consequently lowers the numbers of natural enemies of other pests it is likely that overall pest populations will increase and therefore an increase in the use of pesticides may be required (Babendreier *et al.* 2003).

The possibility of hyperparasitoids is worthy of consideration as they can have a negative effect on the success of a primary parasitoid as an effective control agent (Luck *et al.* 1981; Holler *et al.* 1993). In North America one of the introduced parasitoids to control gypsy moth itself has 16 species of hyperparasites causing from 50-90% mortality which obviously has knock on effects to the control of the pest (Bourchier and Nealis 1992; Eichhorn 1996). Cryptic-hyperparasitoids which are morphologically very similar/identical to the primary parasitoid or facultative hyperparasitoids which give rise to progeny that can develop as either primary or secondary parasitoids make the problem even more difficult if not impossible to deal with.

A final consideration with regard to the application of bio-control programmes is the ability to assess the dispersal patterns of either introduced or indigenous parasitoids. This obviously has its difficulties especially due to the size of the parasitoids which makes topical marking complicated. Another alternative is external self marking with pollen (Lavandero *et al.* 2004). Resin based dyes applied to crops have also been effectively used to mark and monitor parasitoids (Schellhorn *et al.* 2004). Trace elements have also been trialled as internal markers (Gu *et al.* 2001) and a recent development in the use of a stable calcium isotope ^{44}Ca to mark parasitoids by the transfer of the marker from host food plant to the host, on to parasitoid and finally through to the parasitoid offspring shows promise (Wanner *et al.* 2006).

1.7 Biological control measures against *Hylobius abietis*

To date *H. abietis* in commercial forestry has been controlled by insecticide application to transplants either before planting by dipping/spraying or post planting spraying. In most cases the transplants can receive both a pre and post planting treatment. The chemical targets the adult weevil and must be ingested to take effect. The adult weevils may cause damage to the transplant before it ingests enough chemical to kill it. In these cases the transplants may not be killed by the attack but often the weakness left in the stem may result in breakage in the wind. It would therefore be more beneficial to control the non damaging stages of the weevil i.e. the larvae, pupae or pre emergent adults. However because of the concealed nature of the pest in these stages it is impossible to effectively treat the concealed stages with

chemical insecticides. This fact coupled with the negative effects of using broad spectrum insecticides has led to a renewed interest in biological control of *H. abietis*. Although natural enemies which prey on *H. abietis* have been recorded and studied to varying extents since the early 1900's (Munro 1914; Munro 1928; Munro 1929) no one agent has been used successfully in the field on a commercial basis, on a large scale.

Generalist natural enemies of course will include some birds which dwell or visit forests. However it is thought that birds are of little importance with regard reducing weevil population densities (Munro 1929). Other insects that may compete for its brood material include *Hylastes* and *Cerambycidae* species. However, it is rare that they have any effect on weevil populations other than in individual stumps (personal observation). Entomopathogenic fungi such as *Beauveria spp* occur naturally and have been recorded killing the larvae, but more commonly the adult stage of *H. abietis*. Mortality in the field attributed to this and similar fungi is usually less than 10% (Henry 1995). This fungus is not specific to *H. abietis* and so introduction of non indigenous species or varieties would have to be extensively tested in lab conditions before use in the field. Of the remaining natural enemies of *H. abietis* only two categories, namely parasitic wasps and entomopathogenic nematodes have shown any promise and within these groups only a few species have potential (Henry 1995).

Entomopathogenic nematodes have received much attention due to the fact that they can search out and kill concealed hosts. Nematodes have been in use on a commercial basis for pests in citrus, glasshouse, turf, mushroom and agricultural systems for a substantial time (Paul 1993; Grewal *et al.* 2005). Culture and application in the form of a drench applied around, or on, the target area is relatively easy. In relation to the control of *H. abietis,* the nematodes *Heterorhabditis downesi*, *Heterorhabditis megidis, Steinernema carpocapsae, Steinernema feltiae* have shown the most promise. The forestry commission in Scotland have laboratory and field tested three commercially available nematodes *S. carpocapsae, S. feltiae* and *H. megidis* and found that *S. carpocapsae* can reduce the numbers of *H. abietis* emerging from pine and spruce stumps by up to 70% (Evans *et al.* 2004; Brixey *et al.* 2006). In trials conducted over 3 years by NUIM, *H. megidis, S. feltiae,* and *S.*

carpocapsae, exotic species to Ireland and *H. downesi* an indigenous species were assessed for their ability to reduce emerging adult numbers. In 2 out of 3 trials *H. downesi* successfully suppressed weevil emergence while *H. megidis* and *S. feltiae* suppressed emergence in 1 trial. Over the 3 years *H. downesi* produced the highest parasitism rates, achieving up to 63% parasitism. The highest rates achieved by the next best were 47% and 49% for *S. carpocapsae* and *H. megidis* respectively (Dillon *et al.* 2006).

Commercial trials of nematodes have been carried out on Coillte lands since 2007 with approximately 340 ha having been treated. The sites have mainly been treated with, commercially available, *S. carpocapsae.* Problems with the production of *H. downesi* have meant that it has only been used on a smaller scale (A. Dillon 2009, pers. comm., 8 Aug). In the 140ha treated in 2007 weevil emergence was reduced by 42%. Damage levels on all sites except where the previous crop was pine was reduced to less than 10%, one year after treatment (Anonymous 2007).

Between eight and ten parasitic wasps are known to attack *H. abietis* (Kenis *et al.* 2004). *Perilitus areolaris,* an endoparasitoid of adult *H. abietis*, was not described until 1985 by Gerdin and Hedqvist in Sweden and was not recorded in the UK until Henry while, studying another braconid species discovered it emerging from field collected adult weevils, circa 1995 (Gerdin and Hedqvist 1984; Henry 1995). It is not thought to contribute significantly to reductions in weevil populations. The ichneumon *Dolichomitus tuberculatus* (Geoffroy) is a solitary ectoparasitoid generalist which is known to cause low mortality rates in *H. abietis* larvae (Munro 1929; Hanson 1943). Probably the most common and promising parasitic wasp of *H. abietis* is the larval ectoparasitoid *Bracon* hylobii Ratzebury (Hym: Braconidae). The species is described in Ratzeburgs's Ichneumon der Forstinsekten in which he also refers to breeding of the wasp by Nordlinger (Munro 1914). Rates of parasitism of 1%, 30%, 47%, 50%, 67% and 90% have been recorded by different authors on a range of pine and spruce sites (Munro 1914; Hanson 1943; Henry 1995; Henry 1999; Brixey 2000; Henry and Day 2000; Henry and Day 2001). The parasitoid is widespread in Europe including the UK and Ireland. Research to date has indicated that the parasitoid may be best suited as part of a broader integrated pest

management solution. Average rates of parasitism recorded to date have not been high enough to reduce *H. abietis* population density below economic levels.

1.8 Parasitic wasps

Ants, bees, wasps and sawflies form one of the largest orders of insects, the Hymenoptera. There are over 115,000 species recorded worldwide but as many as 1,000,000 may exist (Sharkey 2007). This order can be further divided into the sub-order Symphyta containing the sawflies and the sub-order Apocrita containing the ants, bees and wasps. Within the Apocrita sub-order two further groups can be roughly distinguished from one another as the aculeate Hymenoptera which includes the social bees, ants and wasps and the parasitic wasps which form the parasitic Hymenoptera (Ronquist *et al.* 1999). It is the latter group that is of most importance as regards bio-control of other insect pests.

In Britain two families of parasitoid wasps i.e. the Ichneumonidae and Braconidae contain approximately 2000 and 1200 species respectively which in turn accounts for almost half of the total number of Hymenopteran species in Britain, a figure standing at about 6500 species. A catalogue of the Irish Braconidae published by the Irish Biogeographical society in 1999 lists 529 known species from the Braconidae family (O'Connor *et al.* 1999). The Braconidae are usually smaller in general than the Ichneumonidae the latter growing up to 17mm long in Britain while the former grows only to about 6mm in length. Of the two families braconids are considered to be the more important as bio-controls and have been successful in a number of control programmes on citrus, sugarcane and cotton (Smith and Bellotti 1996). One reason for this is that many are host specific which make them more suitable to control a target pest. This specialisation also makes braconid species an important monitor species as an indicator of biodiversity. A recent study into biodiversity in Ireland (Ag-Biota) found 170 genera of parasitic wasps of which 10 had been previously unrecorded (A. Anderson 2007, pers. comm., 20 July). One parasitic wasp from Ireland has been successfully used in a bio-control programme in New Zealand. The wasp *Microctonus aethiopoides* (Irish wasp) sterilises the Cloverroot weevil *Sitona Lepidus* which is a major pest of white clover in NZ agriculture (Gerard *et al.* 2008).

1.8.1 *Bracon hylobii* history and life cycle

Bracon hylobii is in the genus *Bracon* and member of the sub–family *Braconinae* of the family *Braconidae* which forms part of the super-family *Ichneumonoidea* of the order *Hymenoptera.* The family *Braconidae* is estimated to contain at least 40000 species worldwide of which ca 1200 have been recorded in Britain while less than 600 are recorded in Ireland. The sub-family *Braconinae* contains over 2000 described species worldwide Approximately 50 species mainly of the genus *Bracon* have been recorded in Britain. These compared to their tropical relatives are small and usually blackish in colour with some orange markings. Some characteristics of the British *Braconinae* are; clearly exerted ovipositors, they attack concealed hosts usually attacking the later larval feeding stages; they are ectoparasitoids and more often than not are strict idiobionts injecting venom which paralyses their hosts either immediately before oviposition or shortly thereafter. *Braconines* are synovigenic meaning they require time post emergence to mature their eggs. The time needed for this to happen can range from days to weeks. Species of *Bracon* have been recorded as either solitary or gregarious however it would not be uncommon for a gregarious species to lay solitary eggs if the host was deemed inadequate for the complete development of further eggs. (Henry 1995). Compared to some ichneumonids, *Bracon* species often have a quite narrow host range but again exceptions occur and some have been recorded on relatively wide range of hosts even from different orders.

The larvae of most *Braconines* have some common features also in that most develop through 5 larval instars before spinning cocoons which, if needed, are used as a means of overwintering. All larval instars use the adapted mandibles to burrow their heads into the host's body; this also acts as an anchor to some extent. Emergence is achieved by the pre-emergent adult chewing first through the cocoon and then if required through the substrate which concealed the host originally (Shaw and Huddleston 1991).

Braconines have been used as successful bio-controls for a range of agricultural and stored products. They are also natural enemies of some forestry pests and it is *Bracon hylobii* which is a natural enemy of *H. abietis*.

Bracon hylobii has not been studied extensively and has only recently received attention due to its possible use as a control measure against *H. abietis*. It was first described in 1884 by a German forester by the name of Ratzeburg. However no detailed study into its morphology or biology was made until the early part of the 1900's when Munro made several observations on both the parasitoid and its host (Munro1914; 1917; 1923; 1928; 1929). This combined with Ratzeburg's description gave the most accurate assessment of the life history of *B. hylobii* until almost 90 years later when Henry carried out further research in collaboration with Day and Faccoli into the parasitoid (Henry 1995; Henry and Day 2000; Henry and Day 2001; Faccoli and Henry 2003). Henry, in his own work found some inaccuracies in Munro's details but it is a combination of these authors along with personal observations that the following description of the life history and morphology of *B. hylobii* is based.

Upon emergence both the males and females are ready to mate. If mating occurs a slight male bias in sex ratio in the progeny will prevail however if the female does not mate all the progeny will be male (Henry 1995; pers. ob). This is a simple trait in many similar insects and can be simply put in the following way. If the sex ratio becomes skewed in favour of the females then there is a need for more males in the population to recreate the balance. So if there are fewer males there is a lower chance of a female encountering a male to mate with and so she lays unfertilised eggs which all develop into males. The female still passes on her genes and also ensures the longer term survival of the local population. Females in some species may control the sex ratio by storing sperm and fertilising or not fertilising eggs.

The general life cycle of *B. hylobii* involves emergence of adults which then mate and possibly feed. This is followed by host searching. If host numbers are low or hard to find the females may decide to leave the habitat in search of a new host habitat and then proceed with individual host searching. A lack of available food resources on site may also entice the females to leave a given site whether hosts are available or not. This will be investigated in this program of research.

When a female narrows her search down to the immediate area in which the host is feeding through antennal drumming, she tends to probe with her ovipositor until the host is detected. If the host is suitable she injects it with venom and lays her eggs. In some species of parasitic wasp the venom paralyses the host immediately where as it is thought that the host is not full paralysed from the venom of *B. hylobii* until a few days after being injected. The general purpose of the venom is to stop the host from attacking or removing the parasitoid eggs or larvae. This is quite understandable in the case of *B. hylobii* as *H. abietis* larvae possess large strong mandibles. The eggs develop quickly into 1st instar larvae at which stage they can move location on the *H. abietis* larva. They will attach anywhere expect for the hardened head capsule. Development through 5 larval instars is reasonably rapid but temperature dependant. Once the larvae are fully grown and the host's body is usually fully exhausted the larvae start to spin silken cocoons. If daily temperatures are still relatively high the larvae will pupate immediately with the pre-emergent adult chewing its way out of the cocoon and then through the bark. However if the temperatures are low at this time the larvae may enter diapause and overwinter as larvae in the cocoons, not emerging until the following May.

1.8.2 Morphology

1.8.2.1 Adults

As with most of the braconid wasps in the UK and Ireland *B. hylobii* is a relatively small wasp with the adult measuring between 3 and 6mm (Munro 1917; Henry 1995; pers. ob) (Figure 1.4). Ratzeburg's description given in Munro 1914 states the size of the adult to be between 1.5mm and 2.5mm, wasps of this size have been observed during work on this thesis but like Henry 1995 the average was found to be closer to 6mm. Very small individuals were noted from big clutches particularly when the host was small. The occurrence of many small individuals in a clutch compared to less large individuals may result in higher total number of wasps in the next generation however this could be mitigated by the fact that larger females tend to be fitter in general, having larger and more eggs available and a higher searching efficiency within patches (Visser 1994).

Figure 1.4. Adult *B. hylobii*

In any case females in a given clutch are almost always larger than the male (pers. ob). Ratzeburg also noted that the female had 31 antennal segments, again in samples observed for this work the number of antennal segments was found to range between 25 to 31 This along with other morphological variations noted lead to further investigations into the possibility of a hyperparasitoid being present through the extraction and analysis of DNA, this will be discussed later. Although Munro 1914 states that he did not observe any hyperparasitoids his observation was made on a small sample of 70 individual cocoons. Henry 1995 did not record any hyperparasitoids during his work either but it is thought to be worthy of further investigation as there is the possibility of a cryptic hyperparasitoid which may only be detectable through molecular sampling.

1.8.2.2 Eggs

A female can lay anything from one to 52 eggs per host however the average is about 5-6 per host (pers. ob). Again this is largely influenced by the egg load of the female and the size of the host (Henry and Day 2000). The eggs are smooth cylindrical tubes of about 1mm in length and 0.2mm in diameter. Once the eggs hatch, usually within a few days at room temperature the larvae proceed to burrow their heads into the body of the host to feed. Both the eggs and larvae can be disturbed and even moved to a new host only to re-anchor and continue development (Henry 1995; pers. ob).

1.8.2.3 Larvae

The larvae develop through 5 instars relatively quickly (Munro 1917) usually leaving the body of the host completely empty, in fact after a period of time the only remaining sign of the host will be the empty head capsule (Henry 1995; pers. ob). If there are large numbers on the host the larvae may run out of food before they have

developed through all the instars. Development will continue without food but the resulting adults will be noticeably smaller than those which had adequate food. Fully nurtured larvae can grow to 6mm in length with a diameter of approximately 2mm (Henry 1995; pers. ob)

1.8.2.4 Cocoons

When development to the 5th instar is complete and after a period of about 24 hours the larva begins to spin a silken cocoon. The larva does this by lifting and rotating its head in a very small circular motion while the head is raised during this process the tail end and most of the body remain anchored and relatively still. It is usually but not always the case that all larvae in a clutch spin their cocoons at the same time. The formation of the cocoon can be completed in approximately one day (Munro 1917; Henry 1995). Recently spun cocoons are more fragile and lighter in texture and colour that older cocoons with overwintered cocoons being of the hardest texture and darkest colour. If there are large numbers of larvae in a clutch and limited space in the feeding chamber the cocoons are usually formed in a tight bunch. Whereas if there are fewer larvae and perhaps more space in the feeding chamber the cocoons may be spaced further apart and not physically touch often only connected by thin strands of silk (Henry 1995; pers. ob).

1.8.3 Host location and oviposition

If there are many suitable hosts in the immediate vicinity of a newly emerged female wasp she has the choice to stay in the area or move away, however if there are low numbers or no hosts she will have to search for a new host habitat. Whether a female emerges into a suitable habitat with suitable hosts is a result of a combination of factors. These include the parasitoid and host biology and the management or development stage of the hosts habitat, particularly in the case of agricultural and forestry crops (Powel and Poppy 2001).

Host location is not a fully understood feature of parasitoid biology as it involves very intricate and subtle cues and responses but it is known that they use various chemical cues to locate their hosts (Nordlund *et al.* 1981; Vet and Dicke 1992; Powel and Poppy 2001) however the stage and or timing of the use of these chemical clues is not fully understood. In general it is thought that parasitoids will first locate hosts

by searching for the hosts habitat/food source (Vinson 1981) perhaps in a similar fashion as the host itself finds the habitat. Once the habitat is found, chemical clues from the host itself probably play a more important role in parasitoid searching (Vinson 1976). In the case of *B. hylobii* this is likely to be volatiles emitted from recently felled coniferous forests and then volatiles emitted from host feeding which are used to search for hosts. Parasitoids have been shown to be preferentially attracted to odours of host damaged or infested plants compared to undamaged or non-infested plants (Vet 1985).

Once the parasitoid wasp has located a suitable habitat more definite searches are made first for an actual breeding site e.g. a stump and then a suitable individual e.g. larva of a certain size. The latter search is probably performed through a combination of smell, vibration, sound and even heat detection (Tumlinson *et al.* 1993; Hanks *et al.* 2001). In *B. hylobii* these cues are picked up by the antennae with which the female drums the area concealing the host (Henry 1995; Faccoli and Henry 2003). Increased parasitism rates have been achieved in a study which exposed a parasitoid to host kairomones (semiochemicals emitted by the host) prior to release (Hare *et al.* 1997).

The final search, and assessment of the host, is done by probing her ovipositor vertically through the bark. While searching for a host with a patchy distribution a gregarious parasitoid like *B. hylobii* will have to make decisions on the number of eggs laid per host as well as the time spent in each patch (Visser 1994) this will influence the rate of parasitism of the host.

Once the host is found and assessed to be suitable oviposition begins. With most idiobionts ectoparasitoids venom is injected into the host prior to oviposition. In some cases this takes effect immediately. However in the case of *B. hylobii* it can be a few days before total paralysis sets in (Henry 1995). Obviously the female wasp is limited to the depth into the bark which she can oviposit by the length of her ovipositor for example, Hanks et al. (2001) found that bark of >17mm provided a refuge for stem boring larvae from attack by two different Braconid wasps they were studying. However weevil larvae deep in the bark of pine and those below the soil

level have been recorded being parasitised by *B. hylobii* (Henry 1995; pers. ob) this would suggest the *B. hylobii* females are able to manoeuvre through loose soil and possibly within the feeding tunnels of the host larvae. If this is the case the female coming in direct contact with the host may be at risk from attack by the host. The larvae of phytophagus insects can defend themselves from attack by wriggling, biting or spitting. A study on the braconid parasitoid *Cotesia sesamiae* which parasitises stemboring larvae were found to have a 25% chance of being killed by the host when attacking it in its feeding tunnel (Potting *et al.* 1999).

Whereas searching can take some time, ovipositioning usually takes a few minutes depending on the depth of the larva being attacked and the number of eggs laid (Henry 1995; Henry and Day 2000; Henry and Day 2001). As the female is quite vulnerable to predation during oviposition it is beneficial to carry it out as quickly as possible.

Climatic factors such as wind and light can also affect parasitoid host location, a study on the braconid parasitoid *Cotesia glomerata* showed that cloudy and/or windy weather could reduce its host searching efficiency (Gu and Dorn 2001). Temperature can also affect the host searching capacity of a wasp, as increased temperature usually increases the activity of wasps (Suverkropp *et al.* 2001).

Female wasps can learn to associate an odour and/or a colour with a food or host source and can switch between host and food searching depending on whether they are hungry or not (Oliai and King 2000; Faria 2005). This can have implications for the success of a bio-control agent in an area with scarce food resources especially if the wasp puts the need for food before the need to find a host (Lewis *et al.* 1998).

Henry (1995), found that in lab conditions *B. hylobii* avoided parasitizing *H. abietis* larvae below 100mg in weight even though there was enough material in larvae of this weight and lower to complete development of at least a single egg. Other studies suggest that given no other choice in host availability or due to an expected shorter lifespan parasitoids will attack hosts which may not have been considered previously (Sait *et al.* 1997; pers. ob).

Another point to consider with parasitoid females is that when they find a host they have choices to make. For instance, a host may be rejected or accepted for several reasons, including its size, life stage, health or the fact that it is already parasitised. These factors may be assessed by the female in the context of the general quality of the hosts in the environment with parasitoids usually narrowing their selection criteria when good quality hosts are available (Strand and Obrycki 1996). The fact that the host may already be parasitised does not mean that it will certainly be rejected (Plantegenest *et al.* 2004) instead superparasitism and or ovicide may occur. Alternatively the female may reject the parasitised host preferring to search for a host which gives her progeny a better chance of survival (Mayhew 1997). Obviously superparasitism will decrease the efficiency of a control agent both through a lower number of total hosts being parasitised and the chance of ovicide occurring. It is not thought that *B. hylobii* superparasitises or carries out ovicide however the occasional occurrence of large clutches, up to 52 on one host in the field (pers. ob.) and the overall low rates of parasitism recorded in the field may suggest the possibility of it occurring. Research on *B. hebetor* (Hymenoptera: Braconidae) suggests that ovicide is more likely to occur as searching females encountered less hosts or more parasitised hosts (Strand and Godfray 1989). This situation could easily occur in the case of *B. hylobii* as the more easily accessible hosts become parasitised the female will have to make the choice to either lay her eggs on an already parasitised host or expend more energy searching for more concealed hosts or new hosts in a different patch.

Adult female parasitoid wasps tend to mark their hosts as they oviposit their eggs this marking pheromone acts a signal to other females that the host is parasitised (Griffith 1971; van Lenteren and Bakker 1975; Hofsvang 1990; Nufio and Papaj 2001). The presence of this marker will affect the number of eggs laid by a subsequent searching female from the point of not laying any to laying fewer than she would have if the host was not previously parasitised. These marking pheromones can then cause an increase in dispersion rate of females away for a host or patch (Roitberg *et al.* 1984).

Some evidence also suggests that female parasitoids are more attracted and spend longer searching host substrate that has been marked by the female hosts during

oviposition (Hoffmeister and Gienapp 1999). The extent to which this applies to *B. hylobii* is unknown and the fact that the marking pheromone is put down at the time of oviposition and that the wasp will not be searching in the site until months later would more than likely mean that the marking pheromone would have dissipated.

1.8.4 Emergence, Diapause and overwintering

Bracon hylobii is widespread in the highlands of Scotland and exposed coasts suggesting it is a relatively hardy creature adaptable to a range of harsh weather conditions (Munro 1914; pers. ob). Overwintered adults emerge as early as the end of April and can continue to emerge up to the middle of November, both the commencement and cessation of emergence is temperature dependant (pers. ob.). Peak activity periods will be investigated to determine coincident phenology with hylobius life cycle. These low numbers of late emerging adults towards the end of the year are probably responsible for a small overwintering population which will not expand until the following year. Adults which emerge towards the end of the year run the risk that their offspring will not have developed and spun cocoons by onset of lower winter temperatures. If the larvae have not managed to spin cocoons by winter they will not survive (Henry 1995).

As temperatures fall a higher percentage of the developing larvae will enter into diapause and will not emerge until the following summer when they will have gone through a prolonged period of cold temperatures followed by a period of sustained higher temperatures. The threshold temperature for induction of diapause is between 10-15°C. At 10°C all the larvae will enter diapause and even at higher temperatures a certain percentage will also enter diapause (Henry and Day 2000).

It is obvious then that the first generation which will be largely made up of an overwintered population will not diapause. However, the second, and more so, the third generation will be more likely to diapause, due to lower temperatures. It would be important to know at what stage exactly diapause is induced and if it is possible that the ovipositioning female can have an influence on the behaviour of the eggs she lays as regards diapause initiation. However it is presumed that it is the larval stage that is influenced by the lower temperatures as it is this stage which overwinters in

the cocoon. If so there is only a relatively small window of 2 to 3 weeks in which temperature can affect the decision to diapause. If for instance there was a very mild autumn followed by a sharp decline in temperatures, it is possible that later generations of *B. hylobii* will not have gone into diapause and will find it difficult to survive in the colder temperatures. This could be one factor in the lag period of *B. hylobii* population increase on newer sites as populations have to build up each year from low overwintered numbers.

Diapause duration in the braconid parasitoid *Asobara tabida* Nees was shown to have a negative effect on both pupal survival and the fitness of post diapause emergent adults (Ellers and van Alphen 2002).

1.8.5 Longevity and fecundity

A general assumption of biological control is that the greater the fecundity of a given parasitoid the greater success it will have as a control agent (Lane *et al.* 1999). The general fitness of a parasitoid is dependent on its longevity, fecundity and its ability to find hosts efficiently (Visser 1994). Some parasitoids received their energy through host-feeding. Others do not feed on hosts but search for other sources of food in the form of honeydew, pollen or nectar (Quicke 1997). The addition of extra floral nectar in target pest habitats has been shown to both increase the longevity and fecundity of parasitoids (Lewis *et al.* 1998; Wratten *et al.* 2003; Jacob and Evans 2004; Bezemer *et al.* 2005; Wanner *et al.* 2006). This can be for a number of reasons. Obviously it supplies the parasitoid with a direct source of energy, but also by adding them to a site, which would otherwise be lacking in flowering plants can result in the adult parasitoid not having to expend as much energy or time searching for food and thus can spend more energy and time parasitising more hosts. If a parasitoid leaves a target pest patch to search for food, depending on how far it travels it may not return, especially if it encounters new host sites in its search. So the addition of flowering plants can have the effect of retaining or conserving parasitoid numbers in the target area (Lewis *et al.* 1998; Faria 2005).

Adult *B. hylobii* does not feed on its host but both its longevity and fecundity can be increased by supplying it with a non-host food supplement (Henry 1995; Henry and

Day 2000). A synovigenic parasitoid, such as *B. hylobii*, may experience short-term egg-load limitation due to lack of feeding however this will usually not result in lifetime fecundity limitation (Lane *et al.* 1999). Henry (2000) found that *B. hylobii* females given honey at 20°C lived on average 24days, approximately 66% longer than those deprived of honey.

Henry (2000) also found that temperature alone had the greatest effect on longevity, fecundity, and generation time and diapause induction. As temperature increased from 10, 15, 20 and 25°C mean survival time, generation time and rate of diapause induction decreased. While oviposition rate and fecundity generally increased with temperature.

The plant quality of the host can have direct effects on sex ratio, size, egg load and longevity of a parasitic wasp (Hunter 2003). Thus in the case of *B. hylobii* one might expect lower populations of the wasp, particularly females when *H. abietis* larvae are developing in poorer quality material such as spruce, larch or fir compared with pine (Thorpe and Day 2002). However a poor host substrate can prolong development of the *H. abietis* larvae and so may prolong the window of opportunity for parasitism (Moore 2004).

1.8.6 Patch exploitation and dispersion

Unlike other Braconid wasps used successfully in bio-control programmes the average rate of parasitism achieved by *B. hylobii* in the field on *H. abietis* is not high enough to control the pest (Henry 1995). Reasons for this may include some of those outlined above with regards to all parasitic wasps, such as the availability of a food source, a lack of synchronicity of host and parasitoid life cycles, host concealment and super or hyper parasitism.

In the field *B. hylobii* always seems to leave a large number of suitable hosts unparasitised. Some studies into patch exploitation and dispersion have found this behaviour in other wasps. The parasitoid *Telenomus busseolae* showed an increase in its tendency to leave a patch containing hosts after each successful oviposition or host rejection (Wajnberg *et al.* 1999). The hypothesis behind these dispersion

decisions is that the female parasitoids are actively sampling their environment while searching for hosts. As they find unparasitised hosts they tend to stay in the patch but as they encounter less unparasitised hosts or as their own rate of oviposition reaches a certain level they are more likely to leave the patch. Another study on *Aphidius rhopalosiphi* (De Stefani Perez) (Hymenoptera: Braconidae) gave similar results as regards reasons for patch dispersion. It was also found that incomplete patch exploitation may have been as a result of the wasp not being able to detect recently parasitised hosts and so would leave a patch early to avoid the possibility of self-superparasitism (Outreman *et al.* 2001). It is possible that something similar could explain *B. hylobii's* incomplete patch exploitation. Exposure to cold temperatures for different periods at second larval instar have been shown reduce oviposition rates, learning ability and distort host discrimination and patch leaving decisions among females of *Anaphes victus* (Hymenoptera: Mymaridae) (van Baaren *et al.* 2005).

2 The objectives of this research project

The primary aim of the thesis is to investigate if *B. hylobii* populations can be manipulated to increase the rate of parasitism in the field to a level at which it contributes to an economic level of pest control.

The research was divided into a series of experiments with objectives as listed below, contributing to an accumulation of data which may address the issue of biological control in the field.

- Does *Bracon hylobii* adult emergence synchronise well with Hylobius life cycle?

- Does *Bracon hylobii* exhibit a feeding preference for particular flowering plants on reforestation sites?

- Do *Bracon hylobii* adults preferentially choose Hylobius larvae from similar host plant species to the ones from which the parasitoid emerged (patch exploitation)?

- Is adult *Bracon hylobii* food source a limiting factor in the rate of parasitism achieved due to the effect on lifespan/longevity?

- What is the variation in field parasitism rates and does it vary according to Hylobius larval food source?

- Can *Bracon hylobii* populations be artificially enhanced in the laboratory?

- Can *Bracon hylobii* populations be artificially enhanced in the field?

- Is hyperparasitism a problem or a factor in the rates of parasitism achieved naturally?

3 Bracon hylobii field emergence

3.1 Introduction

Little is known about the timing of *B. hylobii* emergence particularly in Irish climatic conditions. Gathering data on the timing of first emergence, cessation of emergence and peaks in emergence rates within that period will provide valuable information on the activity of *B. hylobii* throughout a season. It is expected that the first emergence on a site in the early part of the season will correspond with the emergence of the over wintered population, any further emergence will be the generations produced by this overwintered population. During the life cycle of Hylobius on a reforestation site the larval stage which *B. hylobii* attacks is present for a prolonged period (Henry 1995; Henry and Day 2001). The timing of both first and final *B. hylobii* emergence will have implications as regards to the period in which the wasp is present on a site in which there are hosts available. It may also be used to ascertain the best timing of an application or release of other biological control agents, such as entomopathogenic nematodes, in a combined integrated pest management control programme of *H. abietis*. Entomopathogenic nematodes have been proven to successfully suppress populations of Hylobius however they also kill the larval stage of *B. hylobii* (Dillon et al. 2006; Dillon et al. 2008), therefore information on the emergence of *B. hylobii* could be used to adjust the timing of nematode application.

Data on first and final emergence and peaks of emergence within the season of *B. hylobii* activity was gathered during the summer of 2005, 2006 and 2007 from both a Sitka spruce (*Picea sitchensis*) and Lodgepole pine (*Pinus contorta*) clearfell, felled approximately 18 months previously, located in Cloosh forest on the west coast of Ireland (See Appendix, Figure 12.1)

3.2 Objective

The objective of this experiment was to gather information on the emergence of adult *B. hylobii* in the field so it could be compared with known information on the life cycle of Hylobius in an effort to assess if *B. hylobii* adult emergence synchronises well with Hylobius life cycle.

3.3 Materials and methods

Traps were placed on both Sitka Spruce (SS) and Lodgepole pine (LP) clearfells, felled approximately 18 months previously. The design of the emergence trap meant that wasps would not be able to enter the trap and so an accurate indication of emergence trends could not be gathered by simply collecting from traps which covered the same stump for the full season. To build a better picture of emergence new traps were placed over new stumps about 8-9 weeks after first emergence. If new sites were available they were utilised, if not the traps were placed on new stumps within the original site. Random non-trap stumps were surveyed on an *ad hoc* basis throughout the season to ensure there were hosts available. Details of trap setup for each year are described below.

The emergence trap consisted of a pyramid of black cloth (weed barrier) supported by bamboo canes placed over a stump. A hole was cut in the side of the pyramid and a threaded waste pipe joiner was attached to it. A sampling container with some alcohol and honey in it was screwed into the joiner and affixed to the side of the tent with duct tape and Velcro. The trap covered the stump and the major roots. The ends of the trap were covered with conifer needles and moss to form a seal, preventing light entering and wind blowing the trap over. The newly emerged *B. hylobii* adults were attracted to the light coming from the clear container in which they were captured once they entered (Figure 3.1)

Figure 3.1. Emergence trap design.

Emergence traps setup 2005

An original trap design failed to capture any *B. hylobii* so the alternative design described previously was used. As a result of the delay these new traps were not set

up until the 28th of June 2005. They were set up on a Sitka spruce and Lodgepole pine site (ca. 18 months post felling) in Cloosh Forest. It was noted at the time that first emergence was most likely missed but that it was worth collecting data for the remainder of the season. At first 15 traps were set up on each site. The containers were collected and changed on a weekly basis. As the original 30 traps, by their nature, prevented any adult *B. hylobii* from outside the trap entering and parasitising larvae within the part of the stump covered by the trap, it was necessary to set up more traps on previously uncovered stumps to gain data on the next peak of emergence. For this reason an additional 10 traps were added to each site on the 29th of July. Again these traps together with the original 30 traps were collected and changed on a weekly basis. Due to time constraints, trapping was discontinued on the 29th of September. The samples from each container were identified in the lab under microscope. The numbers of *B. hylobii* captured were plotted against time.

Emergence traps setup 2006

To ascertain the timing of first (overwintered) generation emergence (2006), traps were set up on two new sites. Again a Sitka spruce and Lodgepole pine site felled approximately 16 to 18 months previously in Cloosh Forest were used. A survey of the stumps on both sites before the traps were set up confirmed the presence of uneclosed *B. hylobii* cocoons. Ten traps similar to those used in 2005 were set up on the 22nd of March. The traps were placed in two lines of five on each site between two windrows. Within the lines the traps were placed over stumps approximately 20 metres apart. The traps were changed weekly. An additional 10 traps were added to each site on the 22nd of June and a further 5 traps were added to each site on the 24th of August. Trapping continued until the 30th of November at which point no *B. hylobii* had been recorded for at least two weeks. The samples from each container were identified in the lab under microscope. The numbers of *B. hylobii* captured were plotted against time.

Emergence traps setup 2007

Data on the timing of emergence of *B. hylobii* were collected during 2007 to reinforce data collected during the 2005 and 2006 season.

Again a Sitka spruce and Lodgepole pine site felled approximately 16 months previously were used. A survey of stumps on both sites before the traps were set up confirmed the presence of uneclosed overwintered *B. hylobii* cocoons. Ten traps similar to those used in the previous seasons were set up on the 12th of March. Traps were placed in two lines of five on each site between two windrows. Within the lines the traps were placed over stumps approximately 20 metres apart. An additional 10 traps were added to each site on the 8th of June and a further 5 on the 10th of August. Trapping continued until the 23rd of November at which point no *B. hylobii* had been recorded for at least two weeks. Traps were changed weekly and the numbers of *B. hylobii* captured were plotted against time.

3.4 Results

Emergence traps results 2005

A peak of emergence on both the SS and LP site occurred on the 28th of July. There was no obvious second peak noticed on either site however there were slight rises in emergence rates during the end of August on the SS site and during the second week of September on the LP site (Figure 3.2).

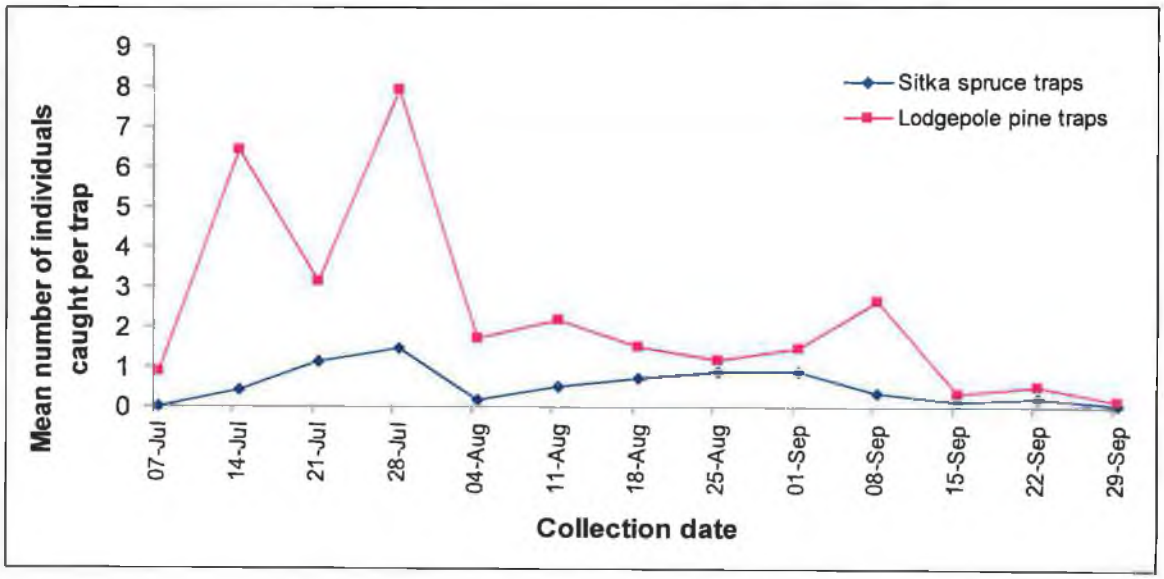

Figure 3.2. Mean number of *B. hylobii* individuals caught per trap during 2005.

Emergence traps results 2006

After 6 weeks of collections (2006), no *B. hylobii* were recorded in any of the traps. To confirm that *B. hylobii* had not emerged some stumps close to the traps were

site a total of 563 *B. hylobii* were captured in the trapping period, of these 348 were male and 215 were female giving a sex ratio of 1:1.6. While on the LP site a total of 1474 *B. hylobii* were captured, of these 995 were male and 479 were female giving a sex ratio of 1:2.1. In total on the two sites combined 2037 individuals were captured in 2006 of which 1343 were male and 694 were female. This gave an overall sex ratio of 1:1.9 (Table 3.1)

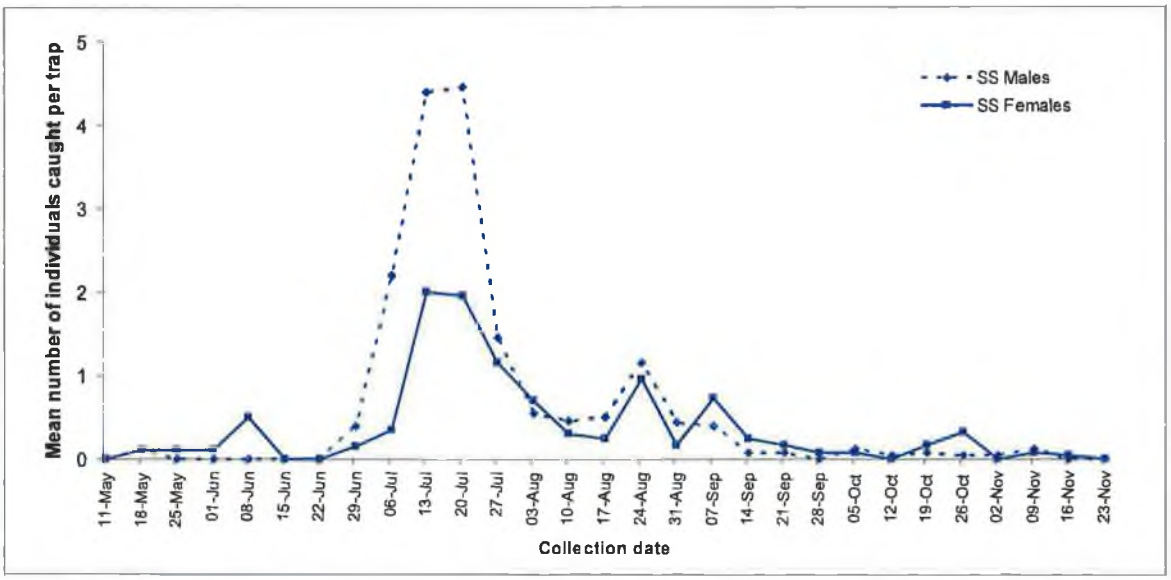

Figure 3.4. Mean number of *B. hylobii* males and females caught per trap on SS site during 2006.

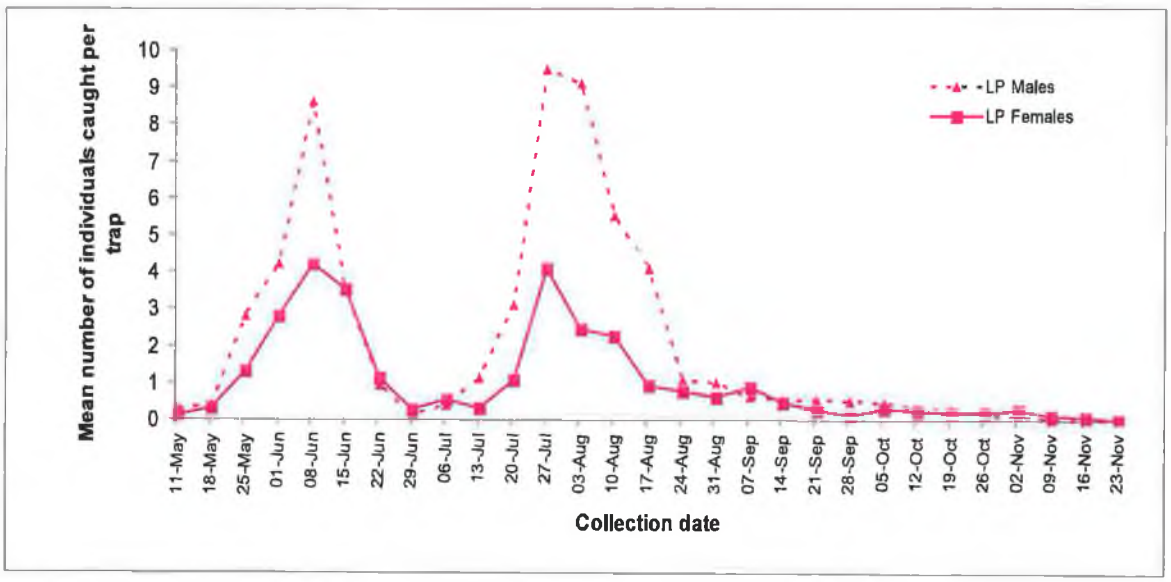

Figure 3.5. Mean number of *B. hylobii* males and females caught per trap on LP site during 2006.

Table 3.1. *B. hylobii* sex ratios in 2006

2006	Male		Female	Total (Male+Female)	Sex ratio
Sitka Spruce site	348		215	563	1.6
Lodgepole pine site	995		479	1474	2.1
Total Male	1343	Total Female	694	2037	1.9

Emergence traps results 2007

Bracon hylobii was first recorded in the traps during the week of the 20th to the 27th of April on the Lodgepole pine and the following week on the Sitka spruce site.

The first peak of emergence occurred on both sites during the week of the 11th to 18th of May. A further major peak occurred on the Lodgepole pine site during the week of 20th to 27th of July. The second major peak of emergence on the Sitka spruce site occurred during the week of the 29th June to 6th of July. As with previous years slight fluctuations in numbers captured were recorded until the end of the trapping period, however no large obvious peaks were recorded (Figure 3.6). No *B. hylobii* were captured after the 9th of November on the SS site or the 19th of October on the LP site.

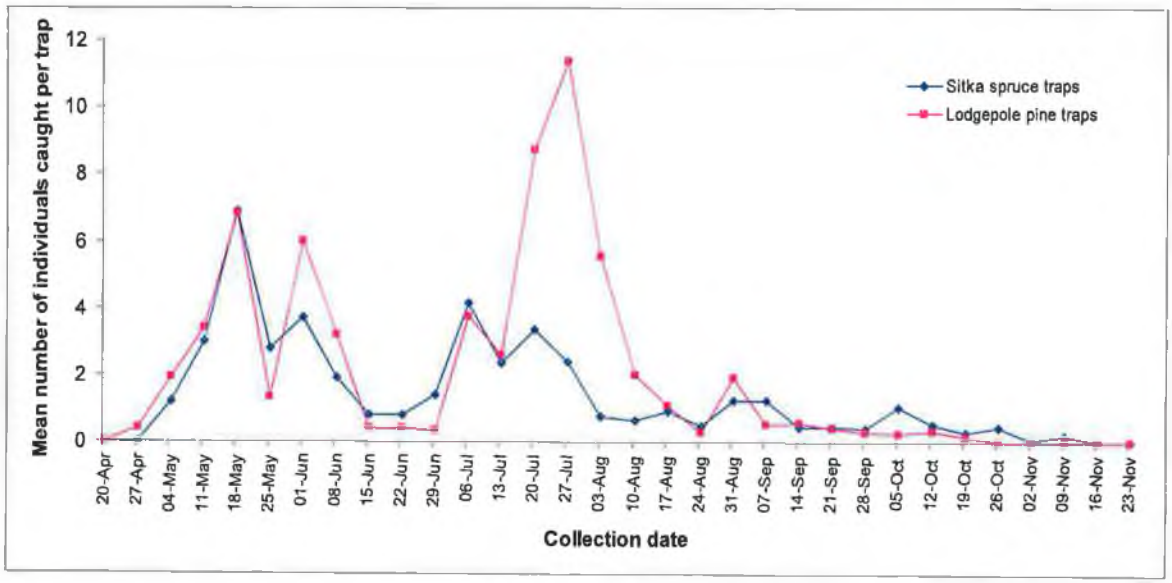

Figure 3.6. Mean number of *B. hylobii* individuals caught per trap during 2007.

Again for 2007 the *B. hylobii* captured in the traps were divided into males and females and plotted against time. In this case male and female numbers did not always peak at the same time. Whereas they peaked at the same time during the first SS peak and the second LP peak, the peaks were slightly offset on the other two peaks. However peak numbers of males were higher than female numbers in all but one peak i.e. the second peak on the SS site (Figures 3.7, 3.8). As with 2006, the number of males caught overall was higher than females but not to the same extent. On the SS site a total of 720 *B. hylobii* were captured, of these 402 were male and 318 were female giving a sex ratio of 1:1.3. While on the LP site a total of 1076 *B. hylobii* were captured, of these 652 were male and 424 were female giving a sex ratio of 1:1.5. In total on the two sites combined 1796 individuals were captured in 2007 of which 1054 were male and 742 were female. This gave an overall sex ratio of 1:1.4 (Table 3.2)

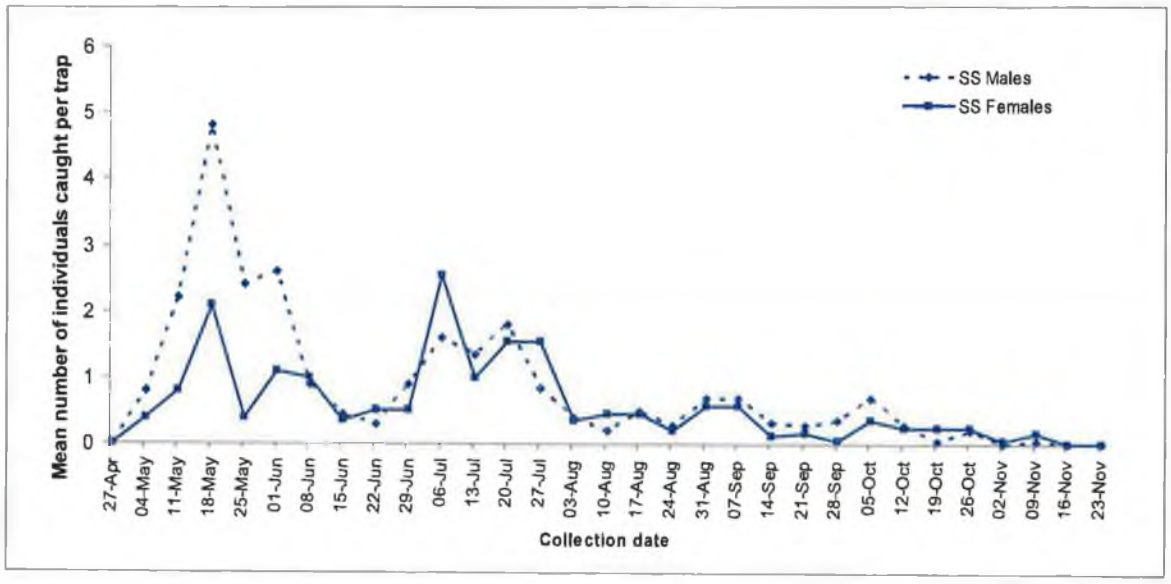

Figure 3.7. Mean number of *B. hylobii* males and females caught per trap on SS site during 2007.

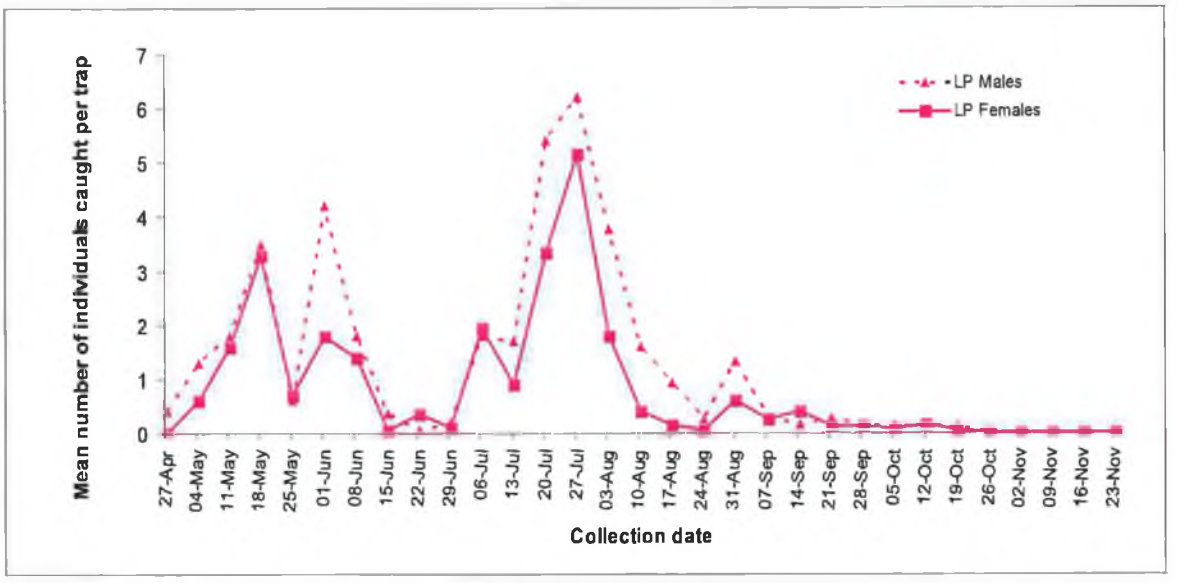

Figure 3.8. Mean number of *B. hylobii* males and females caught per trap on LP site during 2007.

Table 3.2. *B. hylobii* sex ratios in 2007

2007	Male		Female	Total (Male+Female)	Sex ratio
Sitka Spruce site	402		318	720	1.3
Lodgepole pine site	652		424	1076	1.5
Total Male	1054	Total Female	742	1796	1.4

3.5 Discussion

The emergence trends of *B. hylobii* were generally consistent across three years of trapping. First emergence occurred in late April to early May and was followed by two obvious peaks of emergence with emergence stopping around the end of October to the beginning of November. These peaks correspond to the emergence of the overwintered population and the generations/populations produced by those overwintered adults. These emergence times generally agree with observations by Henry (1995). The gradual tailing off of emergence observed in the first generation by Henry was also confirmed in this research. However the timing was different, in this research there was a prolonged tailing off up to the beginning of November

whereas Henry noted it finishing as the end of August, at which time he states all collected parasitoids had entered diapause. The difference may be accounted for in the fact that Henry conducted his research on field collected larval and cocoons held at field temperature (15°C) in the lab whereas this research was based on emergence in the field. This research indicates a longer cycle of emergence than previously recorded.

Due to the reliance on an unsuccessful trap design in the earlier part of the 2005 season both the timing of first emergence (overwintered generation) and the peak of that emergence was missed. However the results from 2006 and 2007 seem to confirm that the peak recorded in 2005 was related to the first generation as second peaks of emergence were recorded in 2006 and 2007 at approximately the same time. On the LP sites the peaks occurred during exactly the same week i.e. the last week of July in all three years.

When results from this, Henry's (1995) and Henry and Day's (2000) research, regarding emergence and diapause of *B. hylobii*, are compared to the average monthly climatic data for Ireland (Met-Eireann 2009) some interesting observations can be made. Diapause initiation and cessation in parasitic wasps is influenced by temperature and photoperiod or a combination of both (Tauber *et al.* 1983; Gordh *et al.* 1999). Henry and Day (2000) found that all *B. Hylobii* cocoons entered diapause at 10°C (LD 16:8h) in the lab. The results of this research found no evidence of emergence after the 16th and 8th of November in 2006 and 2007 respectively. The average highest mean daily temperature in these months was 11°C and 10°C respectively (Met-Eireann 2009). The induction of total diapause in the field at these temperatures concurs with what Henry and Day (2000) found in the lab.

The exact temperature or period of a certain temperature needed to break diapause is not known for *B. hylobii* (Henry and Day 2000). It is known that a period of increased temperature after a prolonged period of low temperatures will break diapause in *B. hylobii* (Henry 1995; pers. ob) and in other parasitoids (Tauber *et al.* 1983; Gordh *et al.* 1999). First emergence occurred during the 4th-11th May in 2006 and during the 20th-27th of April in 2007. The highest mean daily temperatures

during these periods was approximately 10°C-12°C (Met-Eireann 2009), suggesting that temperatures around these values, after a period of cooler temperatures, are enough to break diapause. However it may also suggest that photoperiod plays an important role in diapause cessation, a possibility which requires further investigation, for *B. hylobii*. As the *B. hylobii* overwinter under the bark the effect of photoperiod would may be one of degree day accumulation as a result of longer days rather that the direct effect of increased light.

The differences in first emergence in 2006 and 2007 may have resulted from the unusually cold March/April in 2006 or the unusually warm April which occurred in 2007 (Met-Eireann 2009). This may have influenced degree day accumulation thus affecting the overwintering period.

The results indicate that there are approximately 7-10 weeks between peaks in emergence of overwintered and first generation populations. There was some evidence of smaller third peaks on all sites after the second peak until emergence stops. However it is thought that these correspond to natural fluctuations in the population rather than a definite emergence of a third generation. A point of interest is that peaks occur over a short 1 to 2 week period with numbers falling back quickly after each peak. This trend was also observed by Henry (1995).

More individuals were captured in the LP sites but this is almost certainly due to the large numbers of hosts available in the LP sites compared to the SS sites. A consequence of this may be the fact that the emergence data was more consistent on the LP sites over the three years.

Henry (1995), confirmed to some extent by this research, found that first generation emergence peaks during late July/earlier August of the second and third season. However a large proportion of Hylobius larvae begin to pupate at this time and so may escape parasitism. If this is the case it confirms suggestions that *B. hylobii* is poorly synchronised with its host. This may also be confirmed from the trapping data, which indicated that *B. hylobii* is active from late spring to late autumn. Results proved that *B. hylobii* emerged up until the 16th and 9th of November in 2006 and

2007 respectively. However as this is only emergence data it is possible that the wasp remains active for longer, at least until killed by lower temperatures. A long window of wasp activity should correlate with a greater rate of parasitism, if the host are available at the right stage of development. In the case of *H. abietis* larval development time can be quite long in the field and can take a year or more (Leather *et al.* 1999). This coupled with overlapping of *Hylobius* generations should mean that hosts are available to the wasp throughout its period of activity. The problem it faces is being on the right site at the right time!! Again the lag period between *H. abietis* infestation and *B. hylobii* colonisation coupled with the timing of host pupation and parasitoid emergence, may mean that a portion of the hosts have developed past the point to which they are susceptible to parasitism by *B. hylobii*.

Although there seems to be two major emergence peaks per year, *B. hylobii* is present in small numbers outside these times. This may be an evolutionary strategy to ensure survival in areas where *H. abietis* has unpredictable emergence times or, overlapping generations.

Previous to the use of the emergence traps described above an alternative design using a yellow sticky trap held on a meter tall post was used in 2004. This trap design proved unsuccessful in the capture of *Bracon hylobii.* However the traps did capture a large number of small-bodied flying insects, many of which were considerably larger than *Bracon hylobii*. This led to the following question: If there are large numbers of hosts present within a very short distance of the newly emerged adults do they actually fly very far from the stump from which they emerged to find a new host? This could be a possible explanation for the low numbers captured on the traps and requires some further investigation.

The mean sex ratio on the sites, over the 2 years of 2006 and 2007, was 1:1.65. This male biased sex ratio could result in lower rates of parasitism i.e. less females present. If each year is examined separately the sex ratio is lower (less females) in the SS site than the LP site. Reasons for this are unclear as female parasitoids have been shown to lay more female than male eggs on larger or better quality hosts (Quicke 1997; Bernal *et al.* 1999). One would presume there were larger and better

quality hosts in the pine compared to spruce which should have given a female bias sex ration in the pine.

4 Attractiveness of Four commonly found Clearfell Floral plants to *B. hylobii*

4.1 Introduction

The success of a non host feeding parasitoid biological control agent can be increased by the enhancement and/or manipulation of the non-host food source of the adults (Landis *et al.* 2000; Wäckers 2002; Wratten *et al.* 2002). In the case of parasitic wasps which feed on non-host food sources such as floral nectar, pollen etc research on different species has proven that sites containing an abundant source of this food source can achieve higher rates of control (Wratten *et al.* 2003). Floral food sources can increase rates of parasitism by the control agent in three ways; firstly there is greater food available for the adults especially females which has been demonstrated to increase both longevity and fecundity (Jervis and Kidd 1986; Eijs *et al.* 1998; Lee *et al.* 2006; Rose *et al.* 2006; Hogervorst *et al.* 2007; Irvin *et al.* 2007; Heping *et al.* 2008; Lee and Heimpel 2008; Witting-Bissinger *et al.* 2008); secondly the presence of this food means the control agent is more likely to spend less time searching for food rather than parasitising hosts; the presence of an adequate food source may decrease the likelihood of the control agent dispersing from the target area completely (Lewis *et al.* 1998; Faria 2005).

Bracon hylobii females do not feed on the hosts in which they lay their eggs (Henry 1995). In laboratory studies adults live longer when allowed access to honey (Henry 1995; Henry and Day 2000), therefore it would be a reasonable assumption to suggest that adults would live longer, with higher fecundity, in the wild should they have access to non-host food such as floral nectar and possibly aphid honeydew.

In their natural habitat such as natural conifer or conifer broadleaved mixed forests *B. hylobii* would have greater access to floral resources than in heavily managed clearfell conifer commercial systems, practiced in many countries including Ireland (Smith *et al.* 2005). The clearfell system by its nature tends to leave an area devoid of much flowering vegetation for some time after harvesting until such time the cleared site is re-colonised by flowering plants. This is due to the fact that little light

will have reached the forest floor from the time of canopy closure to final felling. It may also be due to trees and machines ploughing up the site and destroying seed banks.

Research has shown that at least some parasitoids species have the ability to learn to associate a reward of host, food or both with some other stimulus like colour for example (Oliai and King 2000). It has also been shown that innate responses/preferences to a food type exist in some parasitoids (Stapel *et al.* 1997).

Studies have also shown that parasitoids can be attracted to different flowers and as such this should be considered where it is intended to add flowering plant diversity to a site (Wäckers 2004; Bianchi and Wäckers 2008)

4.2 Objective

The objective of this experiment was to test if *B. hylobii* exhibit a preference for flowering plants commonly found on recently felled reforestation sites. A preference for a particular flower may aid in a strategy of site manipulation to enhance wasp retention on site.

4.3 Material and Methods

A survey of flowering plants was carried out on 13th June 2005 on reforestation sites approximately 12 months post felling. Six sites were surveyed covering two soil types namely a peat and a mineral soil. On each site an area of 10m X 20m was surveyed and a visual assessment of the most abundant flowering plants was made. All flowering plants were recorded. In addition roadside plants adjacent to the site were also recorded. A wide variety of flowering plants was recorded.

Foxglove (*Digitalis purpurea*), rosebay willowherb (*Epilobium angustifolium*), marsh willowherb (*Epilobium palustre*) and buttercup (*Ranunculus*) were among the most common plants recorded. Flowers from these four plants were tested in a choice chamber for their attractiveness to *B. hylobii*.

A choice chamber constructed of Plexiglass measuring 90cm by 80cm by 30 cm was used to test the attractiveness of the flowering plants to *B. hylobii.* The main chamber contained 4 smaller internal chambers to hold each flower being tested. Each internal chamber was 20cm by 30cm by 30cm. The internal chambers were opened to the front and had small holes covered with organza drilled in the back to allow air from electric fans to pass through the chamber from back to front. The flowers were held in each chamber in glass jars. The front of the main chamber was open but covered with organza, the wasps were introduced to the middle of the front of the main chamber through a hole in the organza that was sealed again by rubber band after the wasps were introduced. The main chamber was placed in the lab in such a way that it received equal light from all sides. (Figure 4.1).

Figure 4.1. Choice chamber design.

Wasps used in the choice experiment were from cocoons collected from a SS site 2 weeks previously on the 11th of July 2005. The cocoons were carefully separated into individual containers (1 cocoon per chamber) so that the wasps, which emerged, could be classified as mated or unmated. The wasps were tested between 24-48hrs after emergence during the week beginning 25th of July 2005. Only unfed wasps were used.

The first experiment tested the attractiveness of the 4 flowers without providing an empty chamber choice. The 4 flowers tested were separated into each of the 4 chambers in the main chamber. Four classes of wasp were tested, mated and unmated males and females. Four wasps from each class were released into the chamber for 20 minutes and then removed before the next set of four was tested. In total 16 wasps were tested for each class of wasp. A constant flow of air was maintained with electric fans, which were used to pass air through holes covered with mesh at the back of the choice chamber. The position of the flowers was changed after each set of 16 wasps. The chamber was wiped with alcohol and air was passed through each time the position of the flowers was changed. The wasps were taken from a pool of wasps of approximately 6 to 10 for each category depending on availability.

A second experiment that contained an empty chamber option was carried out using males and females which had been allowed to mate. Ten wasps were released first (5 male and 5 females) this was repeated 3 more times. Buttercup was left out of this experiment as it was thought to be the least attractive from the results of the first experiment.

4.4 Results

In the first experiment there seemed to be slight preferences within each class for a particular flower and buttercup seemed to be the least attractive to the wasp (Figure 4.2). However when the results were combined there was no obvious preferences for any of the flowers tested. A chi-square analysis of the data confirmed that there was no significant difference in the choices made in any of the classes tested or when the results were combined (Table 4.1)

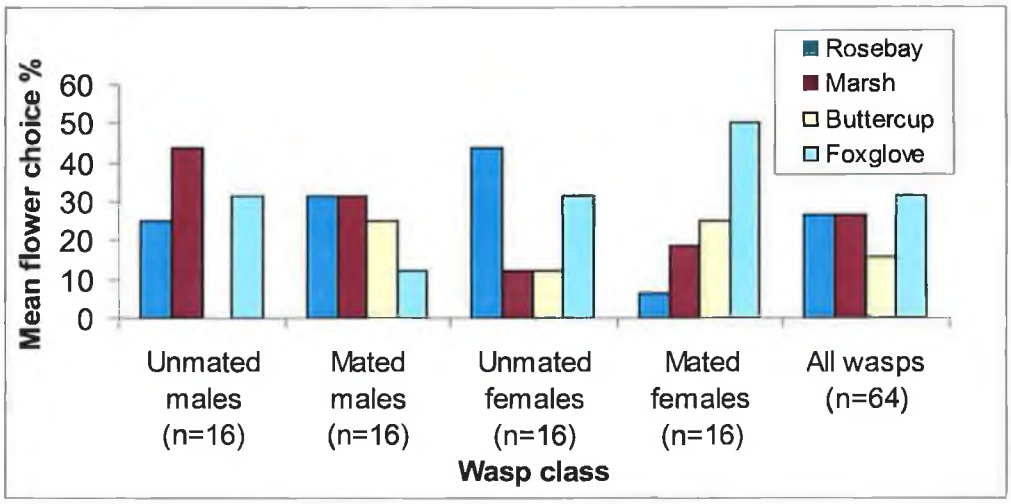

Figure 4.2. Flower preference amongst the four different classes tested.

In the second experiment there seemed to be a preference for the empty chamber for the males and for Marsh willowherb for the females. However when the results were combined there was only a slight preference for Marsh willowherb over the empty chamber (Figure 4.3). A chi-square analysis of the data confirmed that there was no significant difference in the choices made in any of the classes tested or when the results were combined (Table 4.1)

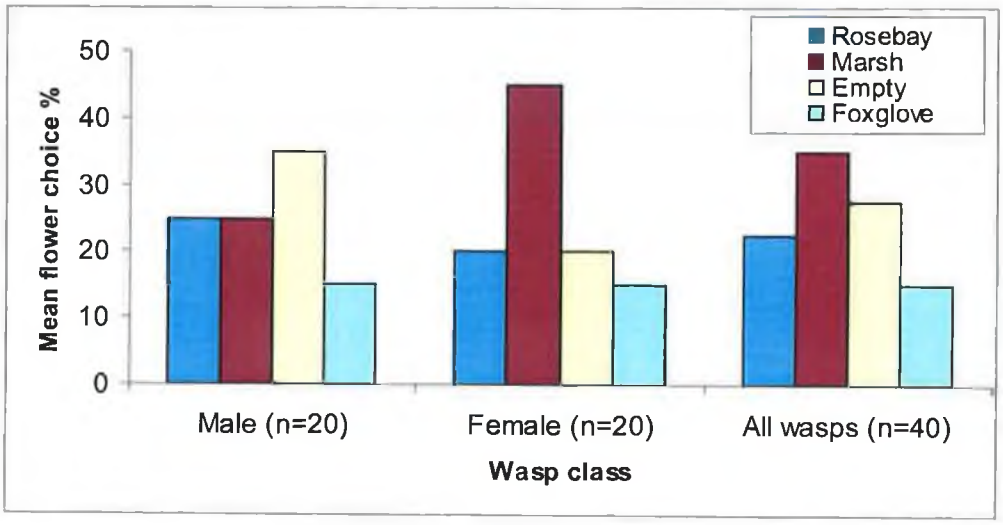

Figure 4.3. Overall preference for each flower of the combined classes.

Table 4.1. Results of Chi-square test

Experiment 1 (4 Flower choice)	Calculated test statistic (x^2)	degrees of freedom	Critical value	Level of significance	Significant/Not significant
16 unmated males	6.50	3	7.81	0.05	Not significant
16 mated males	1.50	3	7.81	0.05	Not significant
16 unmated females	4.50	3	7.81	0.05	Not significant
16 mated females	6.50	3	7.81	0.05	Not significant
64 combined males/females	3.38	3	7.81	0.05	Not significant
Experiment 2 (3 Flower+one empty choice)	**Calculated test statistic (x^2)**	**degrees of freedom**	**Critical value**	**Level of significance**	**Significant/Not significant**
20 mated males	1.60	3	7.81	0.05	Not significant
20 mated females	4.40	3	7.81	0.05	Not significant
40 combined mated males/females	3.40	3	7.81	0.05	Not significant

4.5 Discussion

In general the broad range of choices amongst the tested wasps would seem to indicate no specific preference but more a reliance on the most abundant food source on site at a given time of year. As *B. hylobii* can be present on a clearfell site from April to November a preference or reliance on a particular flowering plant would be a disadvantage. A parasitoid with a long period of activity, such as *B. hylobii,* will obviously have to adapt and find food from different plants as they develop over the season. The type of flowering plant available to overwintered population could be different to the first generation population. In laboratory studies *B. hylobii* has been shown to have a lifespan of approximately 40days (Henry and Day 2000), if this is also the case in the field adults of a single generation may also come across different

flowers as they bloom. This would suggests it would be beneficial to a parasitoid with a long season of activity to be attracted to a variety of plants rather than perhaps a plant of a certain structure or colour. In the case of *B. hylobii* it may learn to associate a certain flower at different times of the year with a greater reward of food. The learning ability of *B. hylobii* was not investigated in this research program but perhaps it is something which deserves attention in the future.

The results of this experiment may be further strengthened by a closer study of plants in the field perhaps both visually and through sweep netting at times when *B. Hylobii* are active on sites.

The portion of mated males and females that chose the empty chamber over the flowers may suggest a tendency to disperse in search of hosts or new breeding areas upon emergence; this was investigated further in other experiment and will be discussed later.

5 Effect of larval food source (tree species) and non-host food on the searching behaviour of adult *B. hylobii*

5.1 Introduction

A large scale inundative release program will involve the mass production of the bio-control agent. In the case of a concealed ectoparasitoid such as *B. hylobii* it is thought that both the substrate that the host is feeding on and/or the substrate which the parasitoid emerges through will affect its searching behaviour when looking for hosts) (Poppy *et al.* 1997; Duan and Messing 2000). Studies on the braconid parasitoid, *Apidius ervi* found that parasitoids which overwintered in alfalfa demonstrated an innate preference to alfalfa plants when searching for hosts (Rodriguez *et al.* 2002). A study on the aphid parasitoid, *Aphidius rhopalosiphi,* found the volatiles absorbed on the surface of the mummy conditioned the emerging adults to search for hosts in environments containing those volatiles (van Emden *et al.* 2002). Others studies have shown that adult parasitoids can show a preference for a particular cultivar of the same species based on which cultivar it was reared on (Poppy *et al.* 1997; von Ellrichshausen 2008). It is possible that *B. hylobii* could exhibit similar innate responses conditioned during development. If *B. hylobii* was to be used in an augmentative release it would be beneficial to assess the extent, if any, of these conditioned responses prior to mass breeding, so that methods and material could be adapted to maximise the potential control on a site by site basis.

Hylobius abietis, the larvae which *B. hylobii* feed on, can breed in a range of conifer species. In Ireland the two most common species in which *H. abietis* breed are Lodgepole pine and Sitka spruce. Another factor in Ireland is that these species are often planted in monoculture. Therefore it would be important to ascertain whether the breeding substrate of *B. hylobii* in the laboratory would need to match the feeding substrate of the hosts on the potential release site.

An olfactometer experiment was set up in the laboratory to test if *B. hylobii* females which emerged from either Sitka spruce or Lodgepole pine logs exhibited a preference for a host feeding on either Sitka spruce or Lodgepole pine.

Another important consideration, when releasing a bio-control agent such as *B. hylobii* into an environment where food and hosts are spatially separated, is whether the agent will put the need for food before the need to parasitise (Lewis *et al.* 1998). This has obvious implications for the success of the control agent. To assess this, newly emerged female *B. hylobii* were also tested for any preference to food over host on emergence.

The tests were carried out on both fed and unfed females.

5.2 Objective

The objective of this experiment was to test if *B. hylobii* adults preferentially choose Hylobius larvae from similar host plant species to the ones from which the parastioid emerged and, if hungry, do they place the need to feed over the need to parasitise. Data gathered would aid in the proper conditioning of wasps in any potential mass rearing/release attempts.

5.3 Materials and methods

For these experiments *B. hylobii* were reared in both a Sitka spruce and Lodgepole pine log. *H. abietis* larvae were transferred from a Sitka spruce and a Lodgepole pine log in which they had been developing from eggs over the last 3-4 months in the laboratory. Larvae developing in a Sitka spruce log were transferred to a new Sitka spruce log in the method outlined in section 8.3.2. The same was done for the Lodgepole pine bred larvae. This was done to ensure adequate numbers of *B. hylobii* were produced for the test. In this case adults were allowed to emerge from the log in the bin and were collected in a plastic tube inserted into the side of the bin.

A 4-arm olfactometer (Figure 5.1 A and C) was designed and used to carry out the test.

The Olfactometer was made from 3 sheets of perspex (30cmx30cnx5cm). One sheet had the shape of the four arms cut out of it. This sheet was then sandwiched between the 2 full sheets to make a sealed inner chamber consisting of a central release chamber with four arms extending from it. Each arm had a small chamber at the end into which the odour source was introduced. The sheets were held together with bolts and secured with wing nuts. This securely maintained the seal and allowed it to be

quickly taken apart for wasp extraction and cleaning. A hole cut in the middle of the bottom sheet allowed a small plastic container to be inserted using a small section of tubing to maintain the seal. Wasps were introduced into the central release chamber by collecting them in the plastic container and inserting it into this hole. An aquarium air pump (model Airvolution AV3), aquarium tubing and a 4 way splitter was used to pump air through an airfilter and then into each of the 4 odour sources. Additional tubing connected each odour source to the odour chambers at the end of each arm of the olfactometer.

The odour sources for the test were 7 *H. abietis* larvae feeding in LP, 7 larvae feeding in SS, honey and a blank chamber (Figure B).

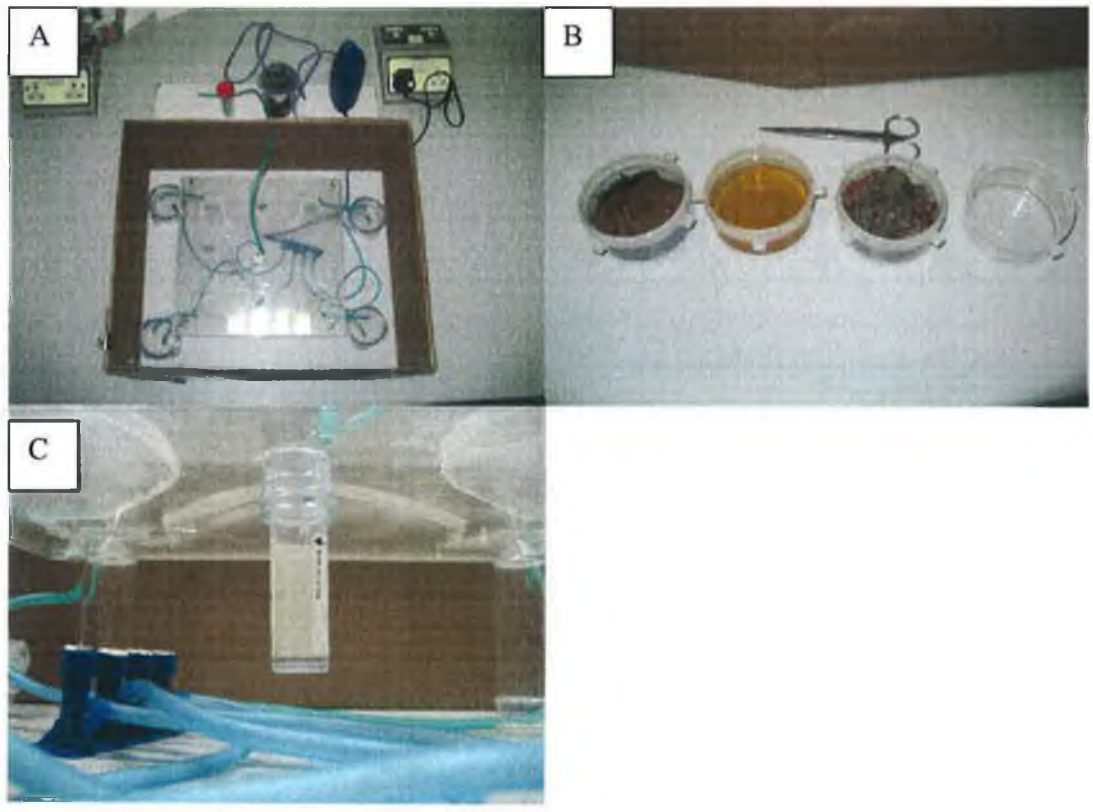

Figure 5.1. 4-arm olfactometer developed to test the searching behaviour of newly emerged female *B. hylobii*

(A=design, B=odour chambers and odours and C=introduction tube)

Experiment 1: *Bracon hylobii* females which had emerged in the previous 24hr and which had access to males but no food source were tested. Forty unfed SS reared

female wasps and 40 unfed LP reared female wasps were tested. Four females were tested at a time and then removed from the chamber. The containers with the odour source were changed to a different arm of the olfactometer after every 4th female. The arms of the olfactometer were also wiped with alcohol after every 4th female.

Experiment 2: *Bracon hylobii* females which had emerged in the previous 24hr were given access to 50% honey/water solution for approximately 2 hours before they were tested. Thirty two SS and LP reared female wasps were tested. The same procedure as above was repeated for this test.

5.4 Results

Unfed:

Honey was chosen a significantly greater amount of times by unfed LP reared females (X^2_3 =14.6, P<0.01). It was again the significant choice when the results of the unfed LP and SS reared females were pooled together (X^2_3 =19.5, P<0.01). Although honey was chosen a greater number of times compared to the other choices by unfed SS reared females alone, it was not significant (Figure 5.2).

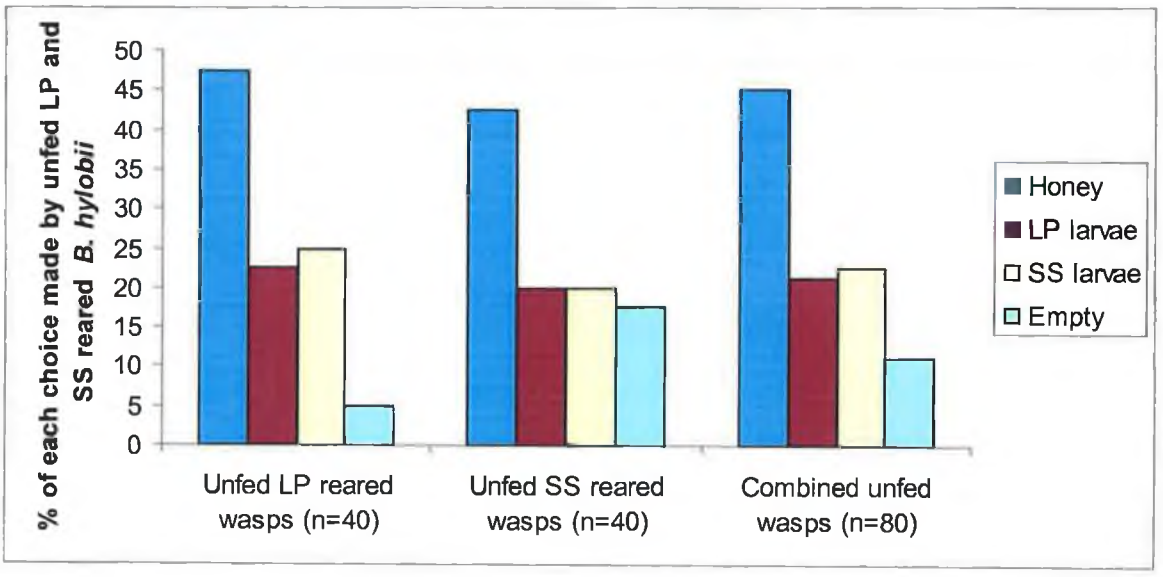

Figure 5.2. Choices made by unfed female *B. hylobii* reared in Sitka spruce and Lodgepole pine when exposed to volatiles from: *H. abietis* larvae feeding on SS and LP bark, honey and an empty chamber, in a 4-arm olfactometer

Fed:

Fed LP and SS reared females did show a preference for LP and SS feeding larvae respectively, however the differences in choices made were not significant (Figure 5.3).

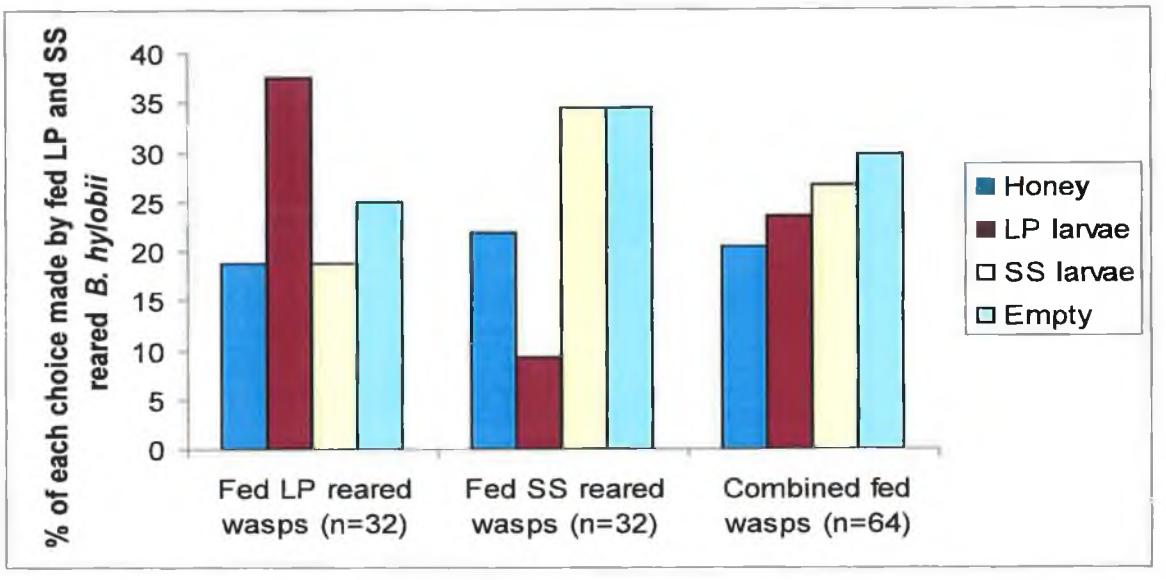

Figure 5.3. Choices made by fed female *B. hylobii* reared in Sitka spruce and Lodgepole pine when exposed to volatiles from: *H. abietis* larvae feeding on SS and LP bark, honey and an empty chamber, in a 4-arm olfactometer

5.5 Discussion

These experiments suggest that newly emerged *B. hylobii* (females) may put the need for food before the need to parasitise. A similar olfactometer experiment on *Bathyplactes curculionis* (Thomson), a parasitoid of the alfalfa weevil, also found that unfed females choose a flower odour over the order of the weevils host plant while the reverse was true for fed females (Jacob and Evans 2001). Research on the braconid parasitoid, *Cotesia rubecula* Marshall, (Hymenoptera: Braconidae) found that while unfed wasps showed an equal preference for flowers and hosts that fed wasp showed a significant preference for hosts (Siekmann et al. 2004). In both field and laboratory studies the parasitoid *Venturia canescens* was able to detect the odours from food or hosts and depending on whether it was hungry or not preferentially choose one over the other. Furthermore it was able to maximise its energy use by detecting food and hosts in combination (Desouhant *et al.* 2005).

If newly emerged *B. hylobii* do actually choose food over hosts the effect will be more pronounced in a habitat, such as a clearfell site, where food is scarce and/or dispersed. They will have to make the decision to disperse in search of food or search for hosts and risk starvation. A model produced in a recent study predicts that hungry wasps should always search for food (Brigitte *et al.* 2006). However if parasitoids disperse in search of food it is not known if they will return to the original emergence/release site. This is further complicated if there are hosts and food present on the site to which the wasp dispersed and by the distance between the new and original site (Lewis *et al.* 1998).

The evidence above indicates that, in a clearfell site, where non-host food is limited (Smith 2005), *B. hylobii* may instinctively leave a site or area in search of food and if so may not return. This may in part explain why attempts to increase parasitism by enhancing populations have not proved successful.

Although the effect of breeding substrate on the choices made by the *B. hylobii* in relation to the feeding substrate of the host were not significant, there did seem to be a preference for SS bred wasps to choose SS fed larvae and likewise for LP bred wasps. As mentioned previously this 'conditioning during development' often occurs in parasitoids and so may need further investigation in *B. hylobii*.

6 Effect of food quality and feeding frequency on the Longevity of *B. Hylobii*

6.1 Introduction

As discussed previously the longevity of non-host feeding parasitic wasps can be increased by the presence of non-host feeding materials such as floral nectar from which they feed. An increase in longevity will mean female adults have a greater window of opportunity to parasitise hosts and therefore it would be logical to suggest that more hosts will be parasitised in areas where adult wasps have increased longevity. Of course this presumes increases in parasitism will be a function of longevity combined with fecundity and dispersal patterns.

As previous experiments seemed to indicate that *B. hylobii* has no preference for certain flowers it may be the abundance and ease of access to this varied food source that is more important. A dispersed sparse floral resource in a target area will mean adult wasps are forced to spend more time and effort finding a food source (Lewis *et al.* 1998; Brigitte *et al.* 2006). This is time and energy lost to the purpose of parasitising hosts.

In many laboratory experiments on parasitic wasps the adults are often fed on an *ad hoc* basis with a honey water solution. Experiments were set up to determine to what extent the longevity of *B. hylobii* is affected by the quality and quantity of an available non-host food source. The effect of water and differing concentrations of honey/water solutions were tested. Two different experiments were carried out. Three treatments of 75% and 25% honey: water solution and water only were tested. In the first experiment the wasps were fed their assigned treatment every 3 days. In the second experiment the wasps were fed their assigned treatment only once.

6.2 Objective

The objective of this experiment was to test if the quality and frequency of feeding would prolong the lifespan of adult *B. Hylobii*. The potential increase in parasitism rates due to increased longevity is discussed.

6.3 Materials and methods

The wasps used in the first experiment were from cocoons collected the previous winter they had been maintained in diapause in the fridge under moist conditions at 5°C for approximately six months at which time they were allowed to eclose at room temperature in the laboratory. As wasps emerged from their cocoons they were assigned to one of the 3 treatments. The wasps were given access to a $1cm^2$ piece of reservoir paper dipped in the treatment at 10am on the morning after emergence. Each wasp was fed in this way on a 3 day cycle with the assigned treatment and held in a petri dish in an incubator at 15°C on a LD 10:14h cycle. In total 57 wasps were tested (3x19). Only laboratory reared mated females were tested.

A second experiment where wasps were given access to the above treatments for one 24hr period following emergence was also setup. Again only laboratory reared mated females were used and held in a petri dish in an incubator at 15°C on a LD 10:14h cycle. In this case each treatment was replicated 10 times (30 wasps tested).
Longevity was measured in days from emergence to death for a particular treatment.

6.4 Results

The mean survival of the wasps fed with water every 3 days was 10.2 days with survival ranging from 4 to 17 days. The mean survival of the wasps fed with 25% honey solution every 3 days was 36.1 days with survival ranging from 3 to 121 days. The mean survival of the wasps fed with 75% honey solution every 3 days was 33.1 days with survival ranging from 4 to 116 days. A significant difference in longevity was found between the water and 25% treatment and the water and 75% treatment (Tukey at P =0.05 following ANOVA, $F_{2,54}$=6.593, P<0.01) (Figure 6.1). No significant difference was found between the 25% and 75% honey solution.

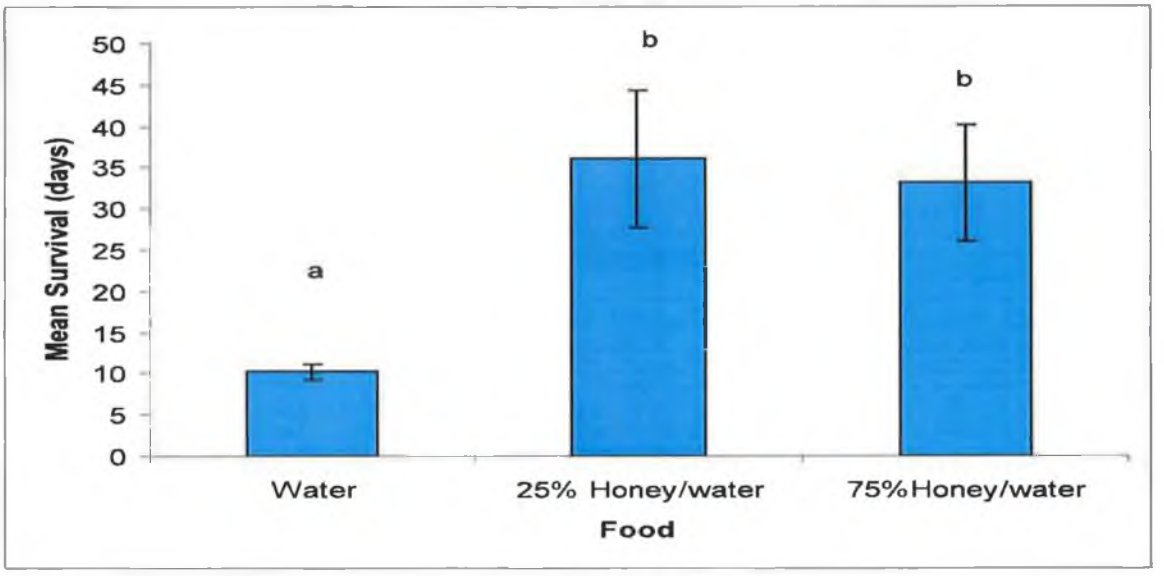

Figure 6.1. Effect of non-host food on the longevity (Mean ± SE) of *Bracon hylobii* (n=19 for each treatment, water, 25% honey and 75% honey, given every 3 days).

Different letters above a column indicate significant differences between treatments (Tukey at $P = 0.05$, following ANOVA, $P < 0.01$).

When the wasps were originally assigned to the treatments in the above test it was noted that some had damaged wings and did not look as healthy as other wasps. However at the time numbers of females available for the test were limited so these wasps were used and recorded as being unhealthy. A total of 15 of these wasps were used (5 in each treatment before more healthy wasps became available. The results of the test were analysed again without these seemingly unhealthy wasps (n=14 for each treatment) the results are as follows.

The mean survival of the wasps fed with water, 25% honey solution and 75% honey solution every 3 days was 11.6, 46.9 and 42.2 days respectively. Survival ranged from 5 to 17days, 7 to 121 days and 13 to 116 days respectively. Again a significant difference was found between the water and 25% treatment and the water and 75% treatment (Tukey at P =0.05 following ANOVA, $F_{2,39}$=10.157, P<0.01) (Figure 6.2). No significant difference was found between the 25% and 75% honey solution.

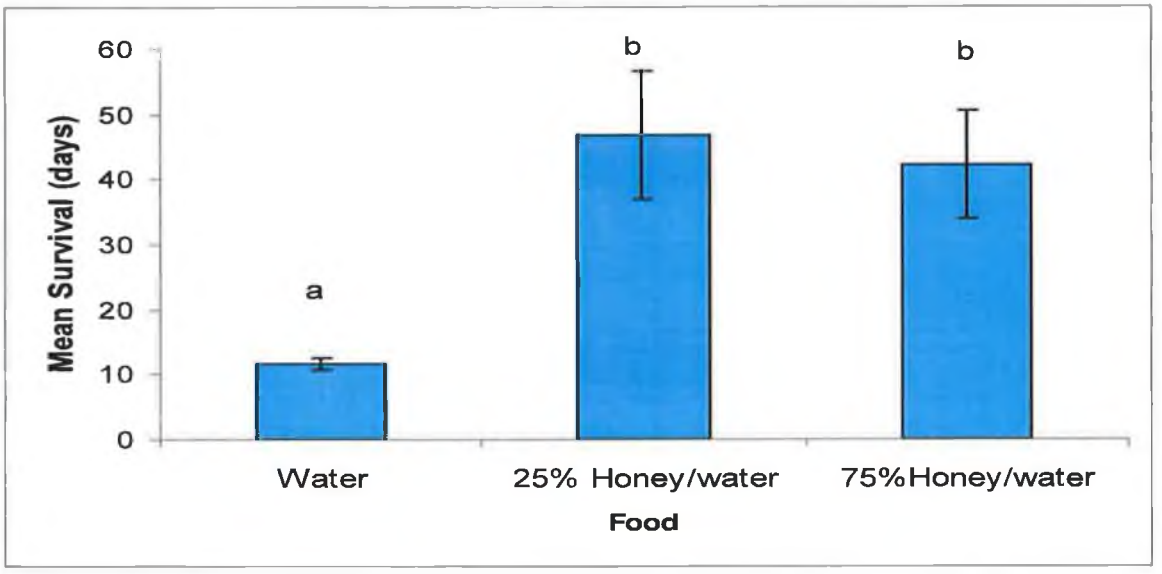

Figure 6.2. Effect of non-host food on the longevity (Mean ± SE) of *Bracon hylobii* (n=14 for each treatment, water, 25% honey and 75% honey, given every 3 days).

Different letters above a column indicate significant differences between treatments (Tukey at $P = 0.05$, following ANOVA, $P < 0.01$).

The mean survival of the wasps fed with water, 25% honey solution and 75% honey solution once was 24.6, 25.4 and 26 days respectively. The differences in the mean survival times were not significant (Figure 6.3). The survival time ranged from 20-28, 17-31 and 20-31 for the three treatments respectively.

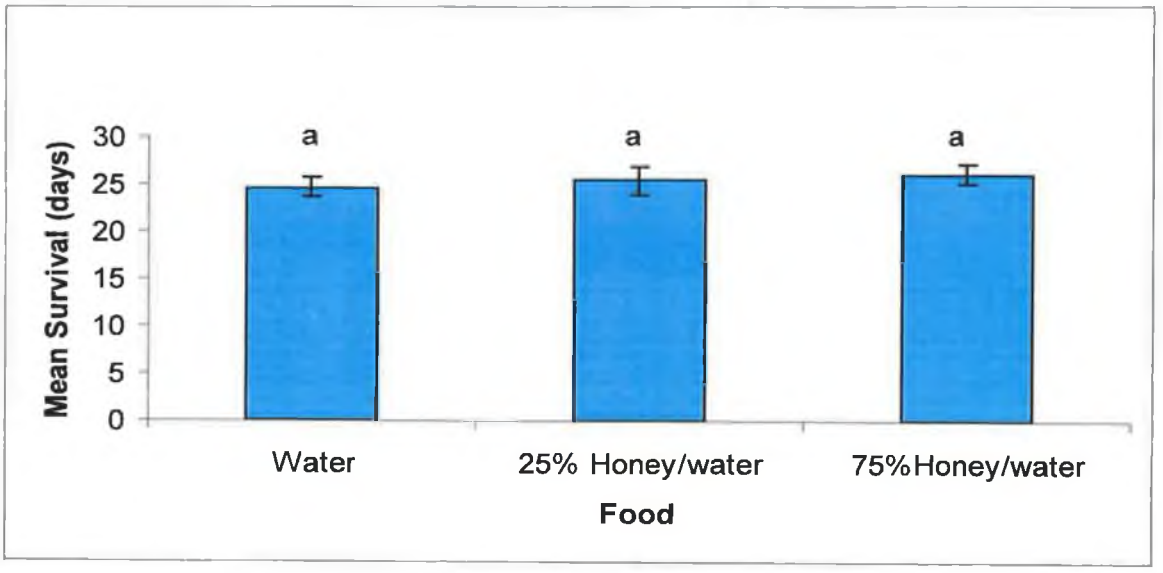

Figure 6.3. Effect of non-host food on the longevity (Mean ± SE) of *Bracon hylobii* (n=10 for water, 25% honey and 75% honey, given once on emergence).

6.5 Discussion

The results from the longevity experiments indicate that the life expectancy of female *B. hylobii* can be increased approximately four-fold when a food of a relatively low sugar concentration is available. A similar experiment on *Meteorus pulchricornis* with continuous access to 30% sugar: water soln. showed it lived six, three and two times longer respectively than unfed wasps, wasps fed once and wasps fed every two days. The experiment also demonstrated that more offspring were produced when the wasps had continuous access to food (Heping *et al.* 2008). Similar to the results of this research, Heping (2008) also found that concentrations above 30 % sugar did not increase longevity. Other experiments on the parasitic wasps *Trichogramma platneri Nagarkatti* (McDougall and Mills 1997), *Mastrus ridibundus* (Bezemer *et al.* 2005), *Diadegma insulare Cresson* (Lee *et al.* 2004), *Cotesia glomerata* (L.) (Lee and Heimpel 2008) found increases in longevity resulting from food quality and frequency of feeding. In his study, Bezemer (2005) also found that the eggs of unfed wasps were significantly smaller than fed wasps and, as Heping (2008) found, fed wasps produced more offspring than starved wasps. The wasp he studied also parasitised fewer hosts when starved. The results of these experiments confirm the positive effect of both longevity and rates of parasitism with the availability of an adequate food source.

The experiment outlined above indicates that the pre-emergent condition of the wasps can affect their life expectancy. Bezemer (2005) also found in his studies that wasps of a greater body size lived longer. This could be a consideration in the field when dealing with overwintered vs. non-overwintered populations. The longevity of the wasps fed only once was unexpectedly high when compared to wasps fed regularly. However this again could be due in part to the condition on the wasps on emergence. The wasps used in the later experiment were noted as being large relative to the ones used in the first experiment. Even so the longest survival time attained by any wasp fed only once was 31days which was just over a quarter of that achieved by the longest surviving wasp (121 days) which was fed regularly. As discussed in previous chapters the available food source on site may be an important consideration in terms of length of searching and reducing probability of adults dispersing from a site.

7 Parasitism rates of *B. hylobii* in field and laboratory conditions

7.1 Introduction

Before any field enhancement of a bio-control agent takes place it is necessary to know the baseline natural rate of parasitism occurring. Information on the location of the agent and its host will yield important information on the ability of the agent to attack the host at different locations within the host habitat e.g. cut stump. Rates of parasitism by *B. hylobii* in the field under natural conditions range from 1 to 90% (section1.7). To ascertain local information for this study stump surveys were carried out. Information on the percent parasitism by *B. hylobii* in Lodgepole pine and Sitka spruce stumps ca. 12-18months post felling was gathered. Some information on the location of *H. abietis* and *B. hylobii* was also gathered. Stump surveys were carried out at 5 reforestation sites.

Research indicates (chapter 1.6) that low numbers of a parasitoid such as *B. hylobii* in the field is a factor when low rates of parasitism are achieved. An inundative release of a bio-control agent in the field is based on the assumption that increasing numbers of the agent in the field will increase the rate of control. It would be important to know how much, or at what rate, an agent should be released so as to optimise the benefit gained for the resources put in. A first step in this process would be to assess the effect of differing rates of numbers released in a controlled environment in the laboratory. A laboratory experiment with the aim of assessing the effect of releasing increasing numbers of female *B. hylobii* into test chambers containing SS and LP logs (inoculated naturally with *H. abietis* larvae) on the rate of parasitism was carried out.

7.2 Objective

The objective of stump surveys in the field was to assess field parasitism rates and to assess the variation in parasitism between different Hylobius larval food sources. The laboratory experiment described here was designed to test if releasing increasing numbers of females in laboratory conditions would increase the rates of parasitism

achieved. This would give reason to assume that increasing numbers released in the field may also increase parasitism rates in the field.

7.3 Materials and methods

7.3.1 Naturally occurring parasitism rates of *B. hylobii*

During 2005 stumps approximately 16 months post clearfell were destructively sampled on 3 occasions. The number of stumps sampled varied due to time and labour constraints. The substrate type of all sites was peat.

The first sampling took place on the 24th of February when 5 Lodgepole pine (LP) stumps were destructively sampled. During the second and third week of March a second set of 20 Lodgepole pine and 20 (results from 19 as one stump incorrectly recorded) Sitka spruce (SS) stumps were sampled. During the third and fourth week of April a third set of 10 LP and SS stumps were surveyed.

The following protocol was used in all stump sampling:

- Before the soil around the stump was removed the distance from the surface of the cut stump to the surface of the soil was measured (Figure 7.1).
- The soil was then removed to expose the main stem and all the roots to a distance of approx 1metre from the main stem.
- All bark, down to the fine roots, in each stump was removed.
- The bark and the underlying stem and roots were examined carefully for the presence of *H. abietis* and *B. hylobii*.
- The condition (alive, dead, parasitised) of each *H .abietis* and *B. hylobii* found was recorded.
- *H. abietis* were recorded as adults, pupae or larvae. An empty pupal chamber with a corresponding exit hole was also recorded as an adult. The presence of *B. hylobii* larvae or cocoons (either eclosed or not) indicated parasitism by *B. hylobii*.

The location of each *H. abietis* and *B. hylobii* found in the February survey were recorded, the distances measured are shown in Figure 7.1. Only the condition

(alive, dead, parasitised) of each *H. abietis* and *B. hylobii* found was recorded in the March and April surveys.

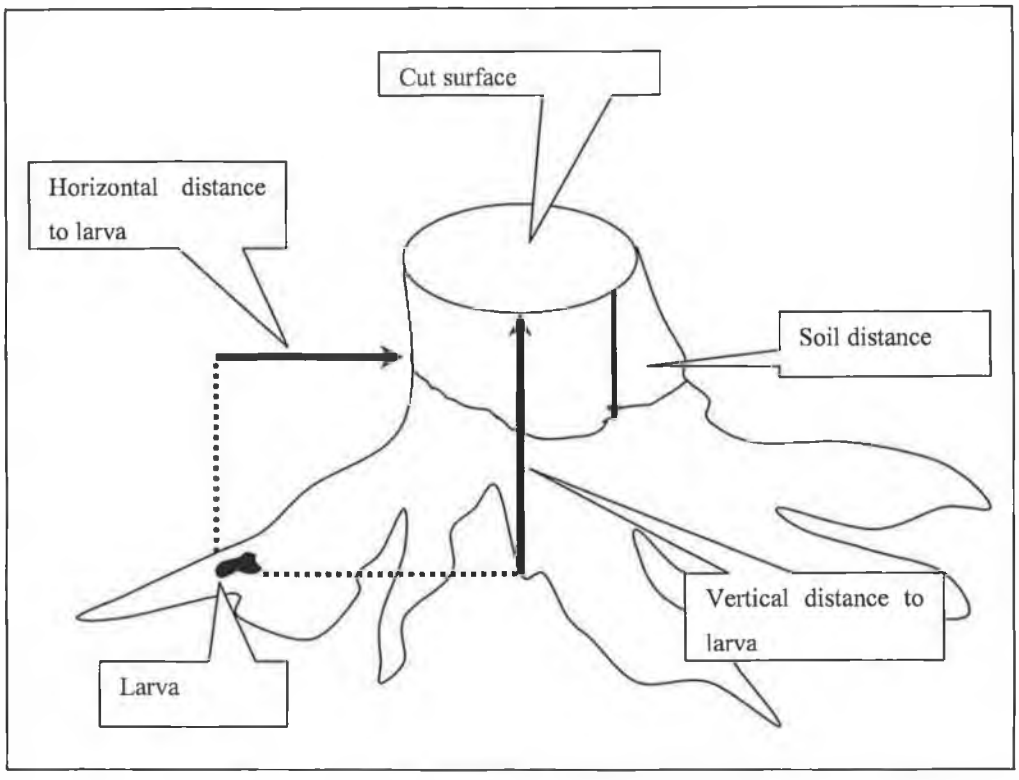

Figure 7.1. Diagram of distance recorded for *H. abietis* and *B. hylobii* located in February stump surveys

7.3.2 Inoculation with differing numbers of females

The test chambers used were standard large plastic dust bins (approx 90L) which were big enough to take 2 cut logs of approximately 50cm in length and 24cm in diameter. The logs were covered up to halfway (approx 25cm) with peat dust. In total 24 of these chambers were used, 12 contained 2 SS logs and 12 contained 2 LP logs. The logs had been inoculated with weevils on the 4th of May, 5 adult weevils were released into each of the chambers. On the 24th of July the 24 breeding chambers were separated into 12 SS and 12 LP chambers. These 2 groups were randomly grouped into 4 smaller groups of 3 chambers each containing 2 logs. A treatment of releasing 1,5,10 or 20 females was then randomly assigned to each of the 4 groups i.e. 1 female was released into each of 3 SS chambers, and 1 female was released into each of 3 LP chambers and so on for 5, 10 and 20 females. The wasps were given a honey solution periodically. The wasps were from cocoons which had been collected

from a Lodgepole pine site approximately 2 weeks previously and allowed to eclose in the laboratory. Females were allowed to mate on emergence and were given access to a 50:50 solution of honey and water soaked in reservoir paper. Once enough wasps had emerged they were assigned to the treatment as described above. All the wasps used in the experiment eclosed within 4 days of each other and were all assigned to the treatments on the same day. After approximately 16 days logs were assessed for percent parasitism. From observations in the laboratory 16 days was long enough to allow *B. hylobii* to develop to pupal stage and short enough so as no larvae would have completed development to adult stage.

7.4 Results

7.4.1 Naturally occurring parasitism rates of *B. hylobii*

The results from the 5 stumps surveyed in February showed an average parasitism rate of 42% with the rate in the individual stumps ranging from 18% to 83%. The average vertical and horizontal distance from the cut surface for the 35 parasitised larvae recorded was 22.99cm and 6.8cm respectively. The vertical location ranged from 12cm to 35cm below the cut surface while the range on the horizontal axis was 0 cm (i.e. on the main stem) to 12 cm. The 11 parasitised larvae which were located on the main stems ranged from a depth of 2cm to 18 cm below the soil surface. Another interesting point which was gleaned from the data was that of the larvae parasitised by *B. hylobii,* almost 82% were located on the south facing side of the stumps. The above details are illustrated in Figures 7.2 and 7.3.

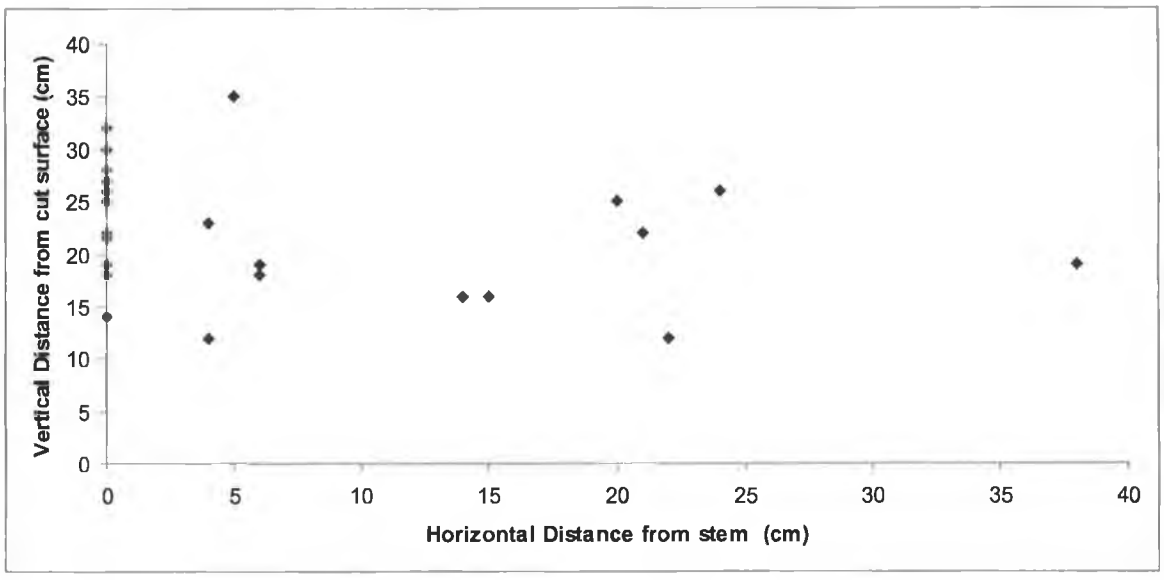

Figure 7.2. Location of parasitised weevil larvae in 5 Lp stumps (ca. 12-18months) surveyed Feb '05 (n=35).

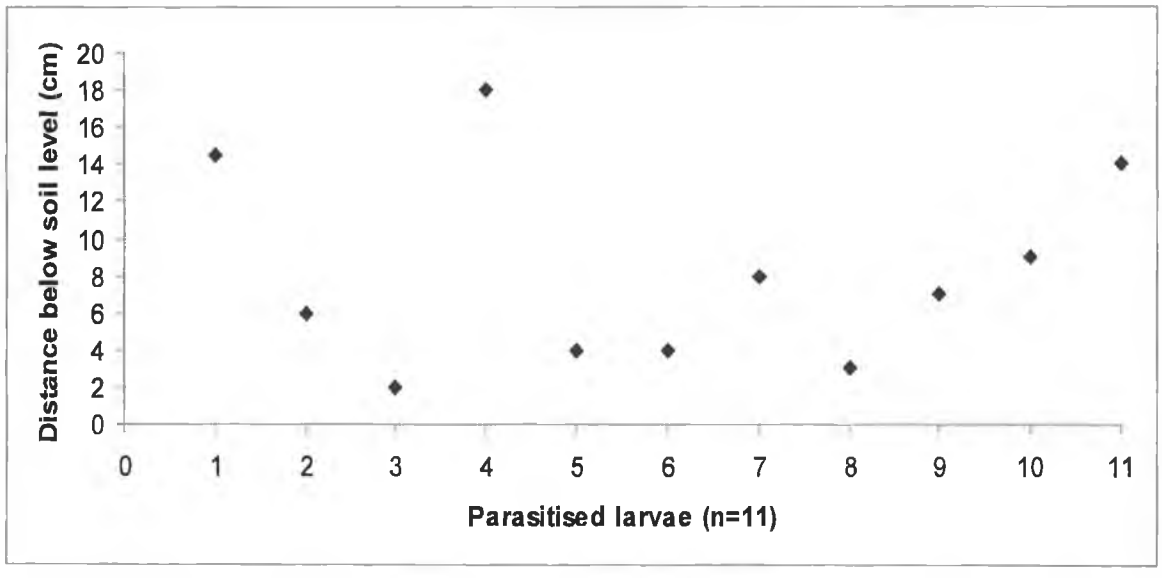

Figure 7.3. Distance below soil level of 11 parasitised weevil larvae on main stem in 5 Lp stumps (ca. 12-18months) surveyed Feb '05 (n=11).

The results from the 39 stumps LP stumps surveyed in March gave an overall percent parasitism by *B. hylobii* of 32% while 19% of the weevil larvae were dead from some other cause. In the SS stumps a percent parasitism rate by *B. hylobii* of 21% was found while 26% of the larvae were dead due to some other factor. The 20 LP stumps contained a total of 348 weevil larvae giving an average of 17 larvae/stump.

The 19 SS stumps contained 146 larvae giving an average of 8 larvae/stump. Of the larvae parasitised by *B. hylobii* there was an average of 5 and 6 cocoons per larva on the LP and SS stumps respectively. All the weevils found were in the larval stage. The above details are illustrated in Figures 7.4 and 7.5.

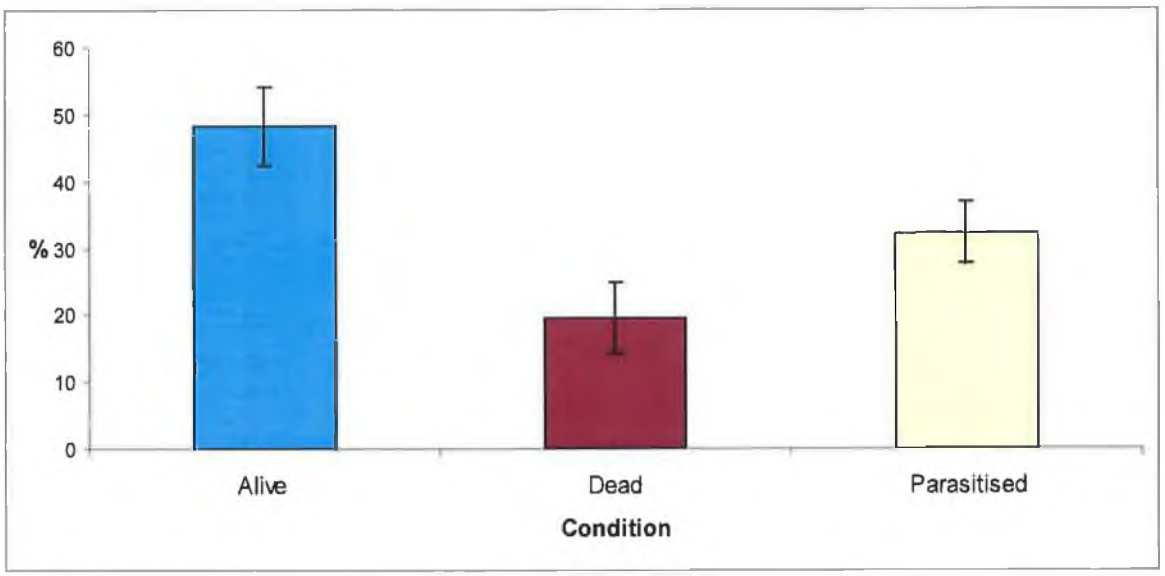

Figure 7.4. Mean (± SE) percent of *H. abietis* larvae alive, dead or parasitised by *B. hylobii* in 20 LP stumps (n=348)

Note: Of the 20 (LP) Stumps surveyed 3 stumps contained no *H. abietis*.

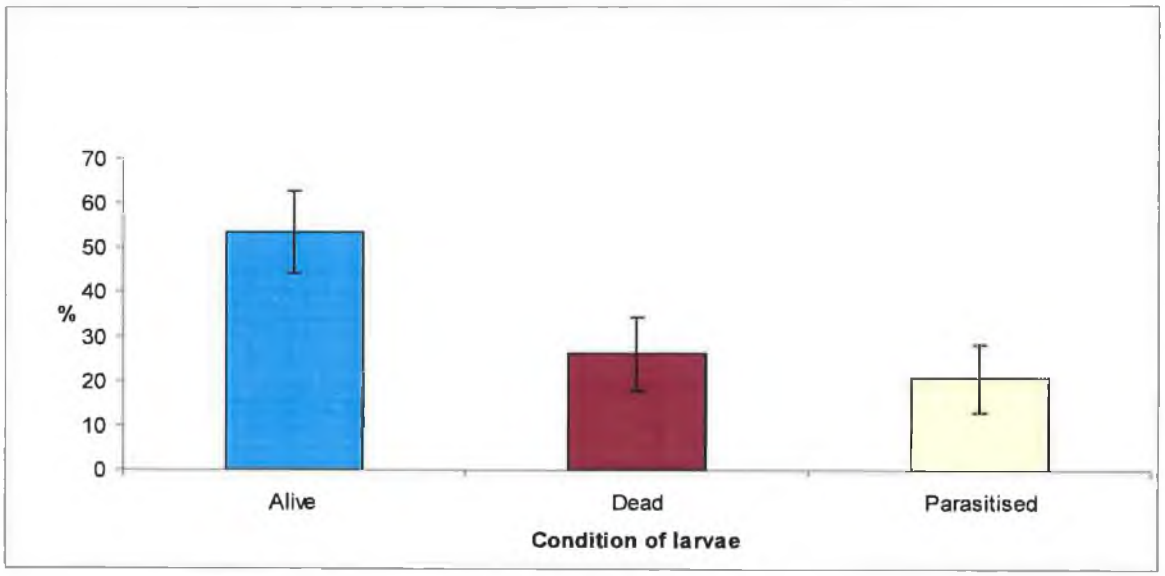

Figure 7.5. Percent (Mean ± SE) of *H. abietis* larvae alive, dead or parasitised by *B. hylobii* in 19 SS stumps (n=146)

Note: Of the 19 (SS) Stumps surveyed 1 stump contained no *H. abietis*.

The results from the 20 stumps surveyed in April showed that the LP stumps gave an overall percent parasitism by *B. hylobii* of 30% while 20% were dead from some other cause. In the SS stumps a percent parasitism rate by *B. hylobii* of 7% was found while 11% of the larvae were dead due to some other factor. The 10 LP stumps contained a total of 10 weevil larvae giving an average of 1 larva/stump. The 10 SS stumps contained 254 larvae and so had an average of 25 larvae/stump. It is worth noting here however that the stumps on the LP site were heavily infested with *Armillaria* which would explain the low numbers of larvae found. Of the larvae parasitised by *B. hylobii* there was an average of 4 and 10 cocoons per larva on the LP and SS stumps respectively. Again all the weevils found were in the larval stage. The above details are illustrated in Figures 7.6 and 7.7.

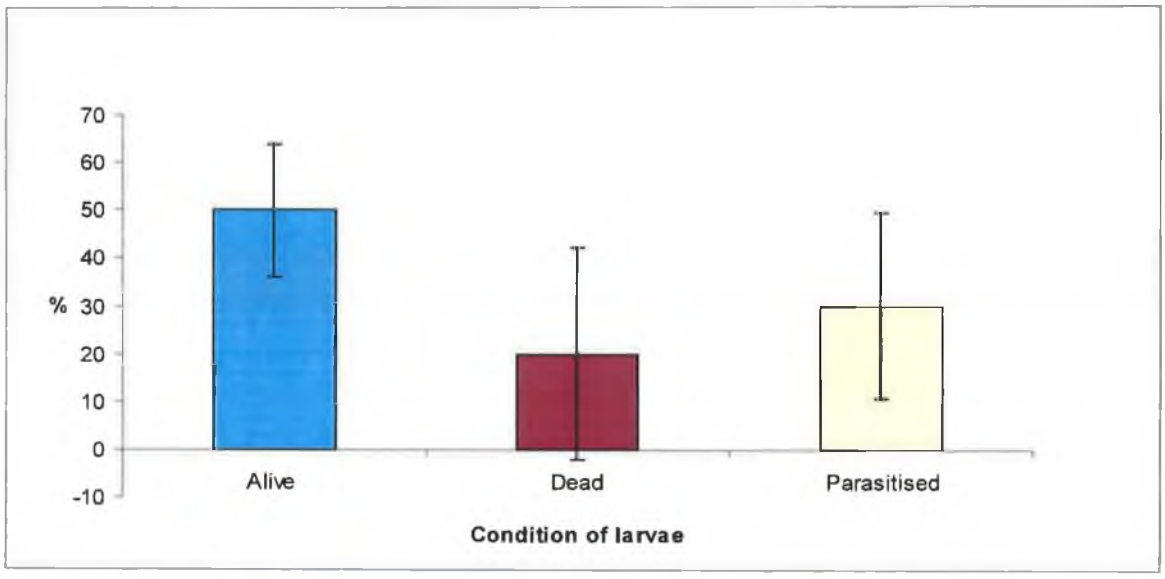

Figure7.6. Mean (± SE) percent of larvae alive, dead or parasitised by *B. hylobii* in 10 LP stumps (n=10)

Note: Of the 10 Stumps surveyed 7 stumps contained no *H. abietis*.

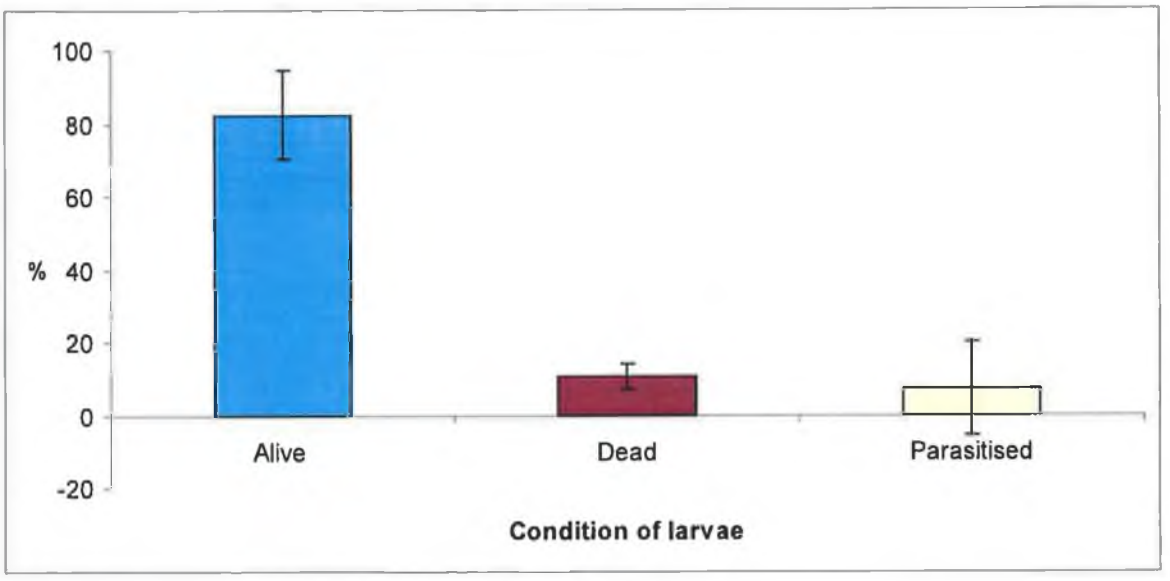

Figure 7.7 Mean (± SE) percent of larvae alive, dead or parasitised by Bracon hylobii in 10 SS stumps (n=254)

7.4.2 Inoculation with differing numbers of females

When the logs were assessed for parasitism the development stage of the weevils in the logs was recorded. This was done to assess the availability of suitable hosts for the introduced wasps. It was assumed that any weevils found as adults or pupae at the time of assessment would have been unsuitable in the 2 weeks in which the wasps were in the chambers. Mean number of total weevils (Adults, pupae, larvae and parasitised larvae), weevil larvae (larvae and parasitised larvae) and parasitised larvae were recorded. The numbers of total weevils were generally consistent across all treatments with the exception of SS1 and SS5 which were higher. The number of available hosts in relation to total weevils was higher in the SS treatments compared to the LP treatments (Figure 7.8).

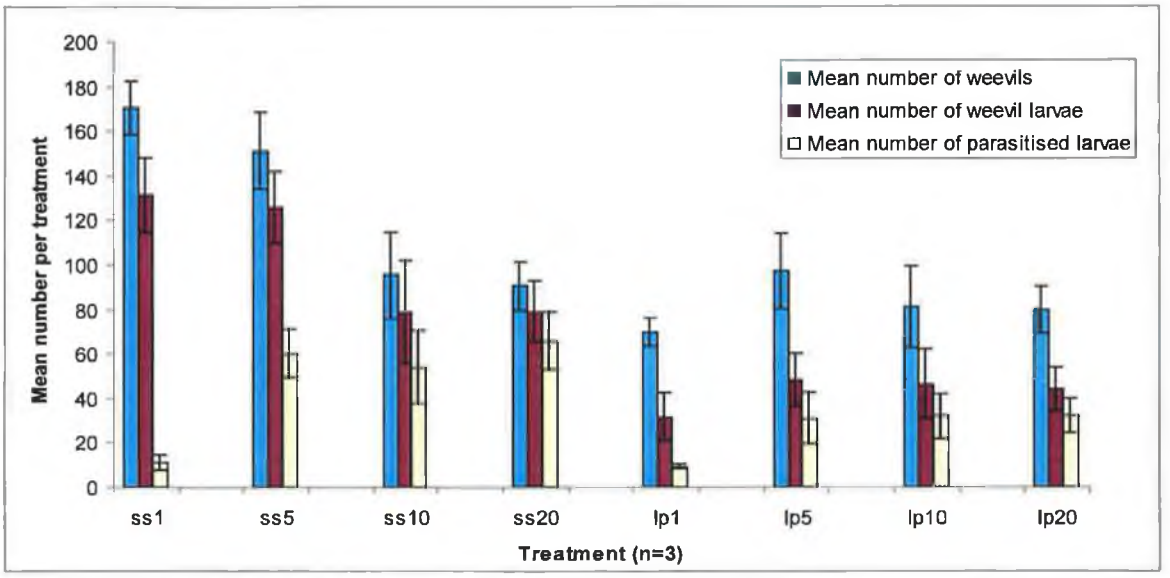

Figure 7.8. Mean (± SE) number of *H. abietis*, *H. abietis* larvae and parasitised *H. abietis* in all treatments

(Note: SS/LP means type of log, 1-20 means number of female *B. hylobii* released)

As each treatment contained different numbers of available hosts the percent parasitism was assessed. The rate of parasitism increased gradually with increasing numbers of females with rates of 8%, 48 %, 68% and 82% achieved in the SS chambers for the 1,5,10, and 20 treatments. In the LP chambers the 4 treatments achieved 34 %, 64%, 71% and 73% respectively (Figure 7.9). Some logs had 100% of the available larvae parasitised. Greater numbers of cocoons were found in the SS logs (Figure 7.10).

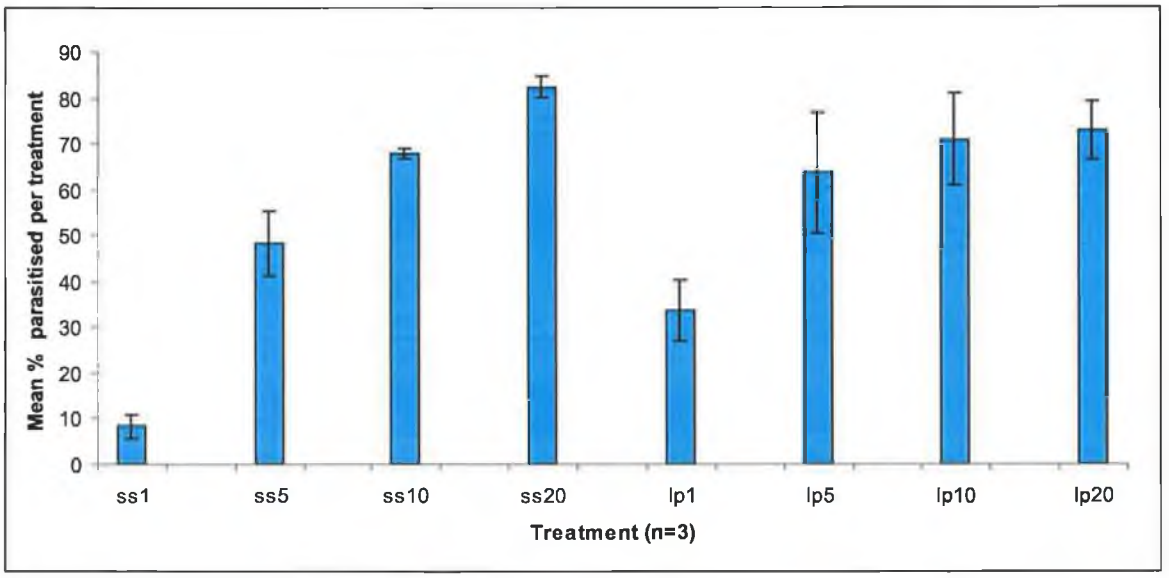

Figure 7.9. Effect of increasing the numbers of *B. hylobii* females introduced on the Mean (± SE) rate of parasitism in the SS and LP stumps.

The difference in mean percent parasitism of the four treatments in the SS logs where n=3 in each case, is highly significant ANOVA ($F_{3,8}$=66.72, P<0.001)
Using the Tukey test, the following treatment means were found to be significantly different at P=0.05: (SS1 and SS10), (SS1 and SS20)

The difference in mean percent parasitism of the four treatments in the LP logs where n=3 in each case, were not significantly different, ANOVA ($F_{3,8}$=3.70, P<0.06).

A two-way ANOVA was used to test the interaction between number of females released and the log type (SS or LP). No significant differences were found between the tree species ANOVA ($F_{1,23}$=2.76,P<0.12) and no interaction was revealed ANOVA ($F_{3,23}$=2.2,P<0.13). However there was a significant difference ANOVA ($F_{3,23}$=23.82,P<0.001) from the effect of increasing numbers of females.

As expected the number of cocoons found in the treatments increased as the number of females released increased. The total number of cocoons found in the treatments range from 133 to 1248 and 149 to 651 in the SS1 to SS20 and LP1 to LP20 treatments respectively. The highest mean number of cocoons in a replicate (i.e. chamber with 2 logs) was in the SS20 treatment in which 416 cocoons were found. This compared to 217 cocoons in the LP20 treatment. The mean number of cocoons per host found in the SS treatments ranged from 4 to 6.3 and 4.7 to 6.8 for the LP treatments, this gave a mean number of cocoons per host of 5 for the SS treatments and 6 for the LP treatments (Figure 7.10 and Table 7.1).

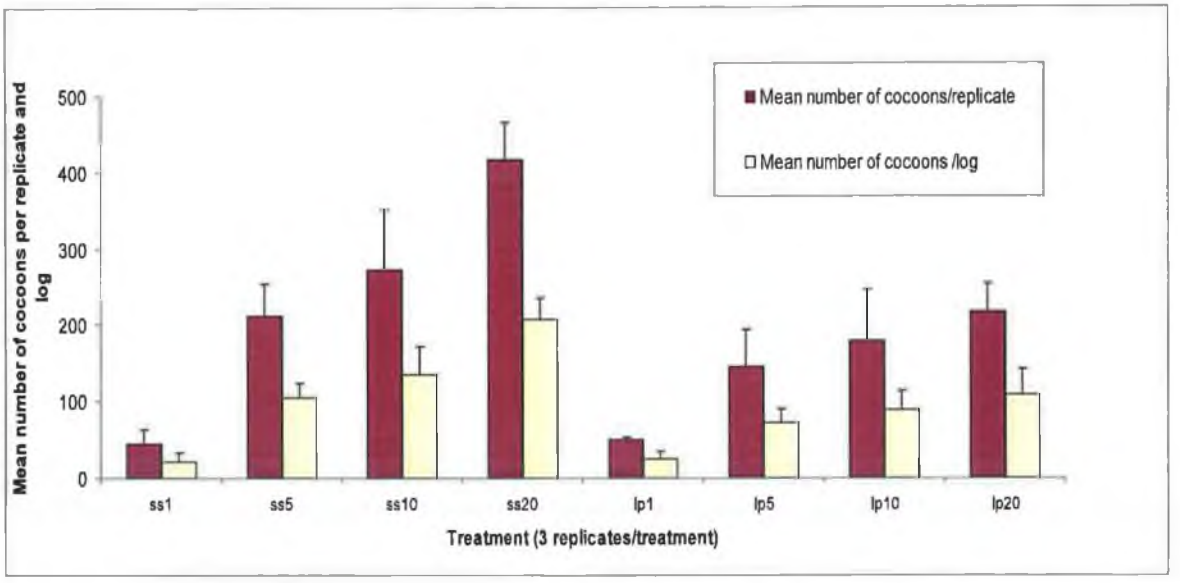

Figure 7.10. Mean (± SE) number of cocoons per group and stump.

Table 7.1. Cocoons details per host and tree species

Group (3x2 logs)	SS1	SS5	SS10	SS20	LP1	LP5	L10	LP20
Total Number of cocoons/treatment	133	636	817	1248	149	436	535	651
Mean number of cocoons/replicate	44.3	212.0	272.3	416.0	49.7	145.3	178.3	217.0
Mean number of cocoons /log	22.2	106.0	136.2	208.0	24.8	72.7	89.2	108.5
Standard error for replicate	19.1	41.8	78.7	48.8	3.8	48.8	66.8	38.0
Standard error for log	11.1	17.6	36.9	27.0	10.1	17.9	24.9	34.2
Mean number of cocoons /host	4.0	3.5	5.0	6.3	5.3	4.7	5.6	6.8
Mean per host for species	5				6			

The number of larvae parasitised above or below the soil level was recorded and showed that the majority of the parasitism occurred above the soil level. As the number of wasp released increased the proportion of larvae parasitised below the soil seemed to increase (Figure 7.11).

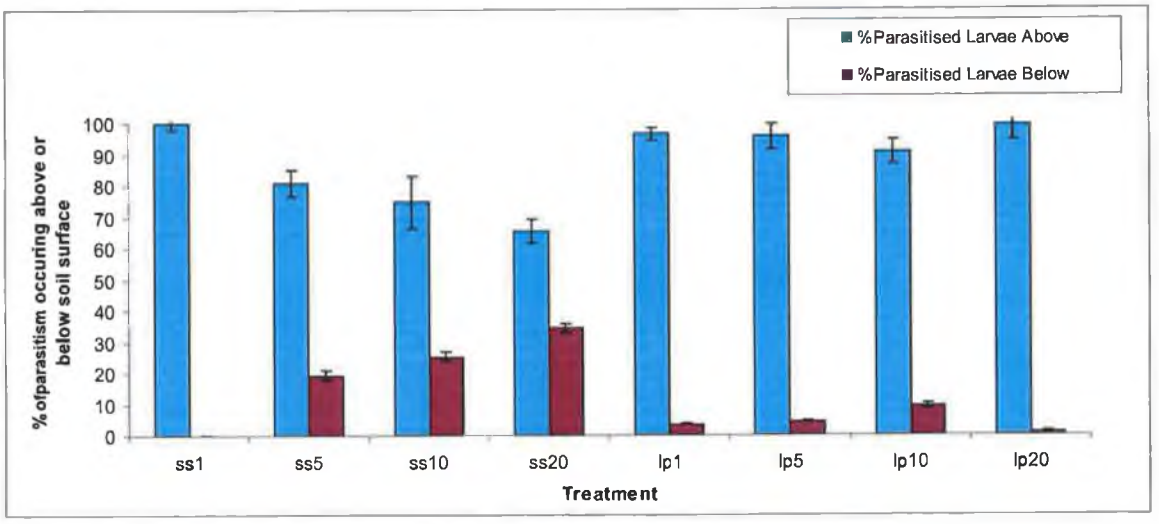

Figure 7.11. Mean (± SE) rate of parasitism in relation to soil level in all treatments.

7.5 Discussion

On field sites it was noted that all stages of development of both *H. abietis* and *B. hylobii* can be present in the same stump approximately 1 year after felling suggesting both overlapping of generations and also quicker generation times for the pine weevil in Ireland than some of the Scottish research would indicate (chapter 1). In general, in the field, parasitism rates were higher in Lodgepole pine sites compared to Sitka spruce sites. It is thought that this may be due to the large number of hosts that are available on Lodgepole pine sites compared to Sitka spruce sites. In the laboratory experiment when large numbers of hosts were available in Sitka spruce logs parasitism rates were higher compared to Lodgepole pine logs and especially compared to the average percent parasitism in Sitka spruce sites in the field. This would also seem to indicate that the higher rates of parasitism in the field in Lodgepole pine are largely due to host numbers. Also, it is important to note that the natural habitat of the host is pine forests. It also indicates that the naturally thinner bark of Sitka spruce compared to Lodgepole pine may be easier for *B. hylobii* to both find and attack hosts.

The rates of parasitism recorded in the field are in line with previous studies on *B. hylobii* (section 1.7). These rates of parasitism by *B. hylobii* are important in reducing the overall numbers of *H. abietis* adults emerging on sites but are not of a

high enough level to reduce plant damage, or mortality, to an acceptable level of 90% survival post year 4 (Henry 1995; Henry and Day 2001).

The location data gathered from the stumps surveyed in February indicate that *B. hylobii* are able to locate and parasitise hosts concealed well below the soil. In this case hosts up to 18cm below the surface were parasitised, this confirms findings from Henry's (1995) research. This is an important point, if *B. hylobii* is to be a successful control agent of *H. abietis* it is vital that it can attack hosts which are often located well below the soil surface particularly on the main stem. However the laboratory experiment seem to indicate that *B. hylobii* prefer hosts above the soil level. As discussed previously an important consideration, in the field, is whether *B. hylobii* will disperse to other sites when hosts above soil level become less available rather than try to find concealed to a greater extent below the soil surface.

As expected, in the laboratory, the percentage rate of parasitism increased as the number of *B. hylobii* females released increased. This was more obvious in the SS treatments than the LP treatments. The levelling off in the rate of parasitism recorded in the LP chambers may have been a host limitation factor as a larger number of the weevils in the LP logs compared to the SS logs had developed past susceptibility to attack. These results prove that in lab conditions high levels of parasitism can be achieved which indicate that there is still potential in the field should factors that negatively impact on it be identified and reduced.

While the overall average cocoons per host was similar in both SS and LP (5 and 6 respectively) there seemed to be a slight trend of increasing numbers of cocoons found per host as the number of females released increased and thus the availability of non-parasitised hosts to searching females decreased. The apparent trend of smaller clutch size when hosts are more abundant would be a useful trait in the field as it only takes one *B. hylobii* to kill a weevil larva and so if weevil numbers were high then individual female wasps should lay fewer eggs per host (Waage 1986) and so potentially parasitise more hosts. On the other hand when the number of adult females on a site increases so too will the parasitism rate, this may cause searching

females to leave the site to increase their chances of finding non-parasitised hosts before a high level of overall parasitism is achieved (competitive displacement).

Results indicate *B. hylobii* seem to prefer hosts above the soil level only increasing parasitism below the soil as overall parasitism increased and thus the availability of host declined forcing females to search below the soil. Reasons for this are probably quite simple and mainly due to the fact that hosts are easier to locate above the soil, as the movement, and thus searching ability of the female wasps would be impeded by the structure of the soil. It would be expected that in the field when searching for hosts below soil level that the wasp would find it harder to move through mineral soil than perhaps a more loosely structured soil such as peat. In the laboratory the bark on the part of the log above the soil remained intact as it was not exposed to the elements as it would be in the field. In the field the portion of the stump above the soil may dry out and become unsuitable for the host quicker than in the laboratory. This maybe result in female wasps having to search below the soil level or, because of the lack of easily located host may decide to disperse from the site entirely. This obviously has major implications for control efforts

8 Field enhancement of *B. hylobii* through inundative and inoculative augmentation

8.1 Introduction

As outlined previously (section 1.7) the natural rate of parasitism achieved by *B. hylobii* in the field is not of a high enough level to reduce plant mortality to an acceptable level. Therefore attempts were made to enhance the field populations, locally, of *B. hylobii*.

The three main methods of biological control have been discussed previously (section 1.6) and included introductions, augmentation and conservation. In the case of *B. hylobii,* a parasitoid which is already present in the wild in Ireland, enhancement of its natural populations in this research program has focused on augmentation and to some extant conservation. The chapter outlines and assesses the different techniques used in an attempt to increase the rate of parasitism by *B. hylobii* through both inundative and inoculative releases.

8.2 Objective

The objective of these experiments was to test if *B. hylobii* populations could be artificially enhanced in the field. If the rate of parasitism could be increased through inoculation in the field, this would potentially give rise to a system where, after an initial intervention, nature would take over and the system would become self sustaining. The possibility of increasing the rates of parasitism achieved in the field through inundative release would justify more research into mass rearing of *B. hylobii.*

8.3 Field enhancement through inoculation

8.3.1 Introduction

Two different methods which centred on the transferral of logs containing viable *B. hylobii* cocoons to clearfell sites with high number of *H. abietis* but low numbers of *B. hylobii* were trialled. The basic principal in the methods used was to allow nature

to do most of the work and therefore reduce the time and labour required. In the first trial, logs partially buried in the field were allowed to become infested by *H. abietis* and then by *B. hylobii*. These logs were then moved to clearfell sites ca. 12-18months post felling as 'Field Infective units' from which it was hoped the rate of parasitism in the surrounding stumps would be increased.

In the second trial logs were allowed to become infested with both *H. abietis* and then *B. hylobii* in the lab under more controlled conditions before being transferred to the field as 'Lab Infective units' into the field. In addition this experiment was used to test if *B. hylobii* could be reared cheaply and in quantity. The task was also expected to reveal whether the tree species in which *B. hylobii* was reared influenced its subsequent host finding, this was also examined and discussed in chapter 5.

8.3.2 Materials and methods

8.3.2.1 Field infective units

Following field assessments seven suitable sites were chosen based on the age post clearfell and potential as *H. abietis* breeding sites. The seven sites were located in Mayo. Two sites were located near Louisburgh, Westport and Ballinrobe with the remaining site situated north of Newport. Locations of the sites are attached (Appendix, Figure 13.1).

During mid August 2004 artificial stumps i.e. logs cut to 75cm, of Lodgepole pine were cut and transported to the seven different clearfell sites. These logs were then buried in each site to create an artificial stump. Ten logs were buried in each of the seven sites, 5 were buried to a depth of about 50-60cm and the other 5 were partially buried on their sides. The only exception to this was one of the sites near Ballinrobe (Ballinrobe site 2) where the parent material was too close to the surface to allow the logs to be buried to 50cm; instead all logs on this site were partially buried. The purpose of this was to allow the logs to become naturally inoculated with *H .abietis* larvae.

On the 13th of April 2005 two logs from each of the seven sites were stripped and assessed for the presence of either *H. abietis* or *B. hylobii*. *Hylobius abietis* larvae were found in all but the Newport site however no parasitised larvae were found. On

the second assessment (2 more logs assessed on each site), carried out on the 13th of May 2005 weevil larvae were again found on all but the Newport site (the Newport site was abandoned at this stage). Two Parasitised larvae were also found on the log from the Louisburgh 1 site. The *B. hylobii* larvae were in an early instar development stage. The first set of logs assessed in March were fully buried logs apart from the one assessed in Ballinrobe 2 as all the logs were only partially buried on this site. The second set of logs assessed in May were partially buried logs. The remaining logs in the field, 6 in each site, were left for a further period of time to allow greater parasitism by *B. hylobii* to occur before finally moving them to new clearfell sites as 'Infective units'.

On the 27th June 2005, approximately 5 weeks after the second assessment the remaining logs were moved to a new SS and a LP site. It was presumed that at this stage the logs had become naturally infested with both *H. abietis* and *B. hylobii* this was confirmed by a partial sample of a log from each location. In this assessment any bark that was removed was stapled back to the log before it was moved. In total 36 logs, 18 to each site were moved. The logs were placed approximately 20 metres apart half way between two parallel windrows. These logs were the 'Field infective units' i.e. logs with *B. hylobii* cocoons in them, moved from other field sites.

Prior to sampling the stumps around the 'Field infective units' all 36 'Field infective units' were collected and stripped in the laboratory, their location in the field was marked and recorded. Ten out of the twelve logs that had come from the Ballinrobe sites were heavily infested with *Heterobasidion annosum*. Due to this fact and time constraints it was decided not to sample stumps around the Ballinrobe logs and also to reduce the remaining 24 'Field infective units' to 20 by random selection. Total weevil numbers including the presence of empty chambers and parasitism by *B. hylobii* was recorded from the 20 chosen 'Field infective units'. It was also noted whether or not wasps had emerged from the cocoons Details of the 20 chosen 'Field infective units' are given in Table 8.1.

Table 8.1. Details of 20 'Field infective units'

Origin	Original Field Log Number	Diameter	Total Weevils including empty chambers	Parasitised by Bracon	% parasitism including empty chambers	Total number of cocoons	Empty cocoons	Full cocoons	Aver No. of cocoons per clutch
L1	1	27	18	15	83.3	50	50	0	3
L2	4	20	8	8	100.0	28	26	2	4
W2	7	22	1	0	0.0	0	0	0	0
W2	8	20	37	12	32.4	118	117	1	10
L2	9	23	14	9	64.3	23	21	2	3
L2	10	24	11	8	72.7	25	22	3	3
W1	15	23	1	1	100.0	11	11	0	11
W1	16	25	5	4	80.0	11	11	0	3
W1	17	20	15	5	33.3	32	30	2	6
L1	18	21	5	2	40.0	2	2	0	1
L1	21	26	2	0	0.0	0	0	0	0
L2	22	22	0	0	0.0	0	0	0	0
L1	25	33	6	1	16.7	11	9	2	11
L2	26	22	22	19	86.4	62	47	15	3
W2	27	21	9	3	33.3	16	16	0	5
W1	29	21	23	18	78.3	99	97	2	6
W2	30	21	7	2	28.6	10	10	0	5
W2	32	17	13	6	46.2	31	31	0	5
W1	34	18	16	6	37.5	45	45	0	8
W2	36	24	9	5	55.6	22	22	0	4

(W1=Westport site 1, W2= Westport site 2, L1=Louisburgh site 1, L2= Louisburgh site 2)

This left 10 'Field infective units' on each site around which stumps were sampled. At first 2 stumps radiating from the selected 'Field infective units' (approximately 2-3 metres away) were sampled (Figure 8.1). Control stumps (i.e. stumps radiating from 'control points' in an area on the site with no 'Field infective units' present) were also assessed. A further 20 stumps selected approximately 10 metres from the each of the 'Field infective units' were sampled on each site

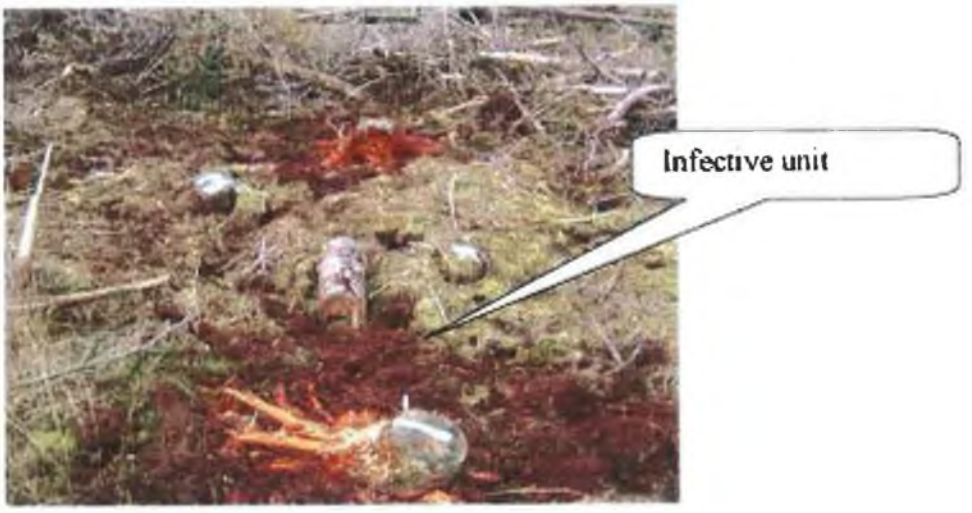

Figure 8.1. Infective unit with stripped stumps.

8.3.2.2 Lab infective units

In the lab newly felled logs (60cm X 24cm approx) of 15 Lodgepole pine (LP) and 15 Sitka spruce (SS) were placed in large black bins, surrounded ¾ of the way up with moss peat and covered with organza (breeding chamber). *Hylobius abietis* adults, collected from billet traps in the field were introduced into the bins. Ten weevils were introduced into each of the 30 bins on the 28th of April 2005. The chambers were kept at room temperature for the duration of the experiment. The weevil larvae were allowed time to develop, before a random subset of one log from both series were debarked and assessed for *H. abietis* success and development stage. On the 15th of June, 48 days after the adult weevils were introduced, one SS log and one LP log were assessed to gauge the development stage of the weevil larvae. These larvae were visually divided into instar development stages based on their relative size.

While a greater number of larvae were found in the SS Log (63) compared with the LP log (50) a higher percentage of the larvae in the LP log were at a more advanced stage of development. The majority of the larvae in both logs were in the early stages of development (instar 1, 2 and 3). On this basis it was decided to leave the remaining logs for some time before introducing any *B. hylobii* adults. During the first week of July one more log each of LP and SS were assessed and revealed that

the *H. abietis* larvae were at a more developed stage and suitable for inoculation by *B. hylobii*.

Bracon hylobii cocoons were collected from the field (SS site in Cloosh) on the 8th and 12th of July. When a sufficient number emerged they were introduced into 26 of the remaining bins. This took place on the 19th of July; 82 days after the adult weevils had been put into the bins. A total of 5 female and between 3 and 5 male wasps were added to each bin. All females had been exposed to males prior to introduction into the bins. The wasps were regularly presented with a 50% honey/water solution. Prior to the introduction of the wasps some soil was removed from each bin to the point that approximately 1/3 of the log was covered with soil as opposed to 3/4 before the wasps were introduced. It was hoped that this would aid the wasps' ability to find hosts.

Between the 4th and 8th of August, 16-20 days after the wasps were introduced and 99-103 days after the weevils were originally introduced, 6 logs (3 SS and 3 LP) were randomly selected and were assessed for the presence of cocoons. The development stage of the weevil larvae was assessed again at this stage. Most were at a late larval stage of development; some had begun to form pupal chambers but only very few were actually pupating (Figure 8.4). The condition of each weevil larva and pupa was also recorded for the 6 logs (Figure 8.5).

As the first 2 logs debarked on the 4th of August contained cocoons it was decided to move the 20 remaining to the field. The 20 logs (10 SS and 10 LP) were moved to the field on the 5th of August 17 days after female *B. hylobii* were introduced into the breeding chambers.
As with the 'Field infective units' described in the previous experiment these logs became the 'Lab Infective units' (details are outlined in Table 8.2).

Five SS and 5 LP logs were moved to a SS site and a LP site. They were placed in areas where no bark stripping or trapping had taken place. The logs were laid out in 2 lines one of 5 SS logs and one of 5 LP logs. Each line was placed in the centre of two windrows. The logs were spaced approximately 20 metres apart.

As with the 'Field infective units', stumps around these ' Lab infective units' were sampled during February 2006 to assess if an increase in percent parasitism among the stumps radiating from these 'infective units' was achieved when compared to stumps in areas where no 'infective units' were placed.

A further 20 stumps selected approximately 10 metres from the each of the 'Lab infective units' were sampled on each site.

8.3.3 Results

8.3.3.1 Field infective units

The average percent parasitism in the stumps around the 'Field infective units' and those in the control area was 22.9% and 23% respectively on the SS site and 42.2% and 43.8% respectively on the LP site. Percent parasitism in the stumps 10m away from the infective didn't differ greatly from the control stumps with rates of 22.7% and 32.2% on the SS and LP site respectively. The infective units had an average parasitism rate of 38.2% and 60.6% on the SS and LP site respectively.

The very similar rates of parasitism in both the stumps surrounding the 'Field infective units' and those surrounding the control points indicated that the 'Field infective units' had no effect on the rate of parasitism in the surrounding stumps (Figure 8.2 and 8.3).

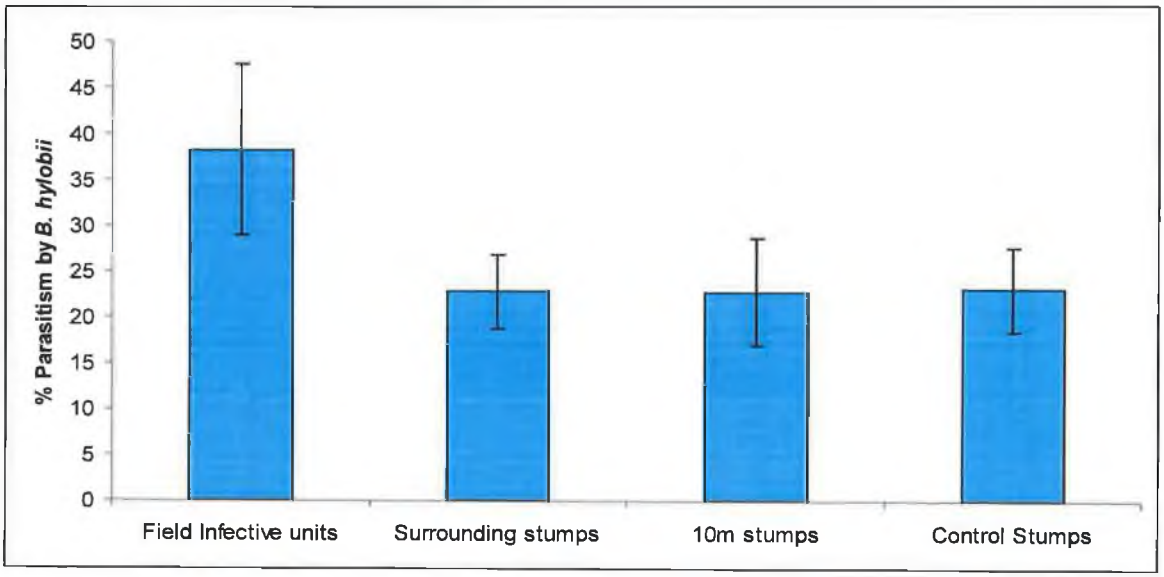

Figure 8.2. Rate of parasitism by *B. hylobii* in stumps surrounding the Field infective units on an SS site

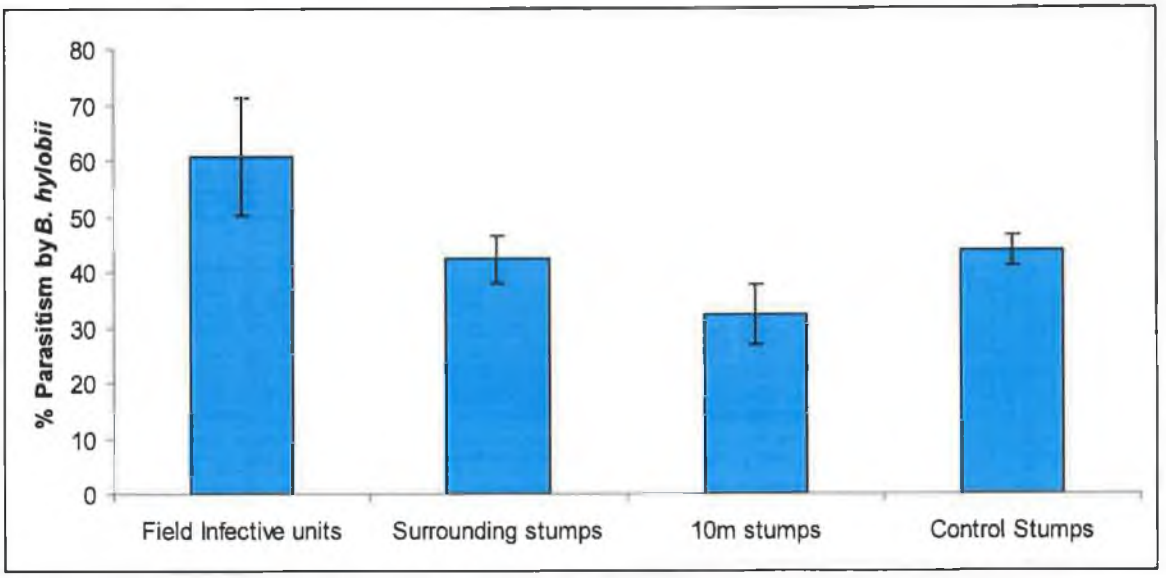

Figure 8.3. Rate of parasitism by *B. hylobii* in stumps surrounding the Field infective units on an LP site

These results were analysed using ANOVA and as expected from initial indications there was no significant difference between the rates of parasitism ANOVA ($F_{2,37}$=0.368, P=0.694) for the SS site and ANOVA ($F_{2,37}$=1.751, P=0.188)

When stripping the 'Field infective units', it was noted that most logs had both empty and full cocoons. However, when the data were analysed there was no correlation between the number of empty cocoons in an 'infective unit' and the rate of parasitism in the surrounding stumps.

8.3.3.2 Lab infective units

Of the 6 logs (3 SS and 3 LP) sampled on the 4th and 8th of August the SS logs had the highest rate of parasitism ranging from 46% to 40% while the LP logs had lower rates ranging from 33% to as low as 8%. It is thought that the lower rate in the LP logs could be due in part to the thicker bark. The development stage and condition of each weevil larva and pupa recorded in the 6 logs sampled is outlined below (Figure 8.4, 8.5 and 8.6).

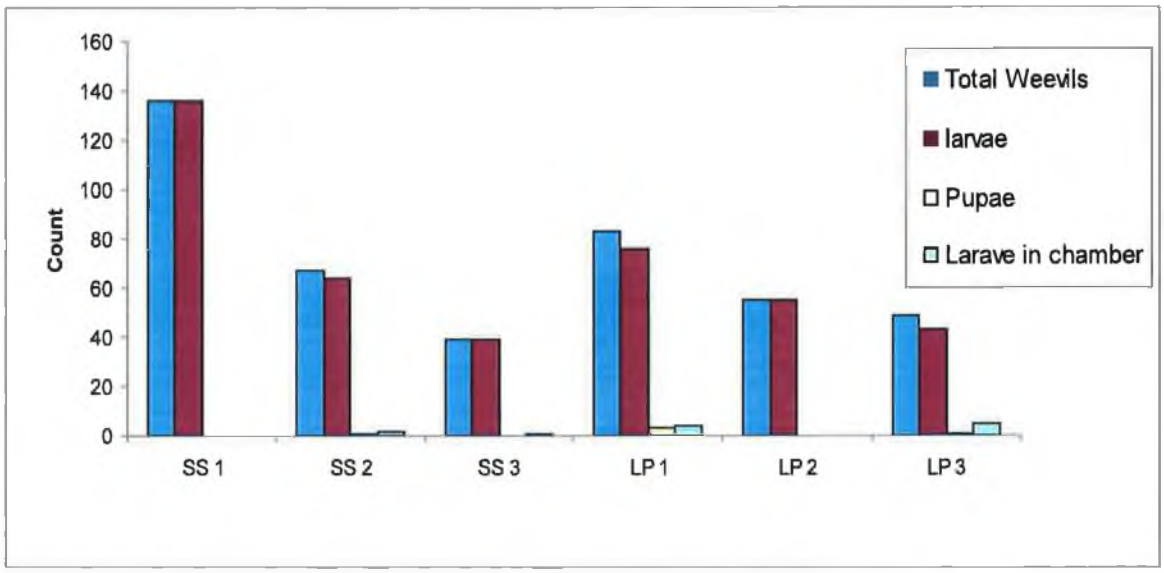

Figure 8.4. Development stage after 99-103 days of *H. abietis* in 3 LP and 3 SS logs

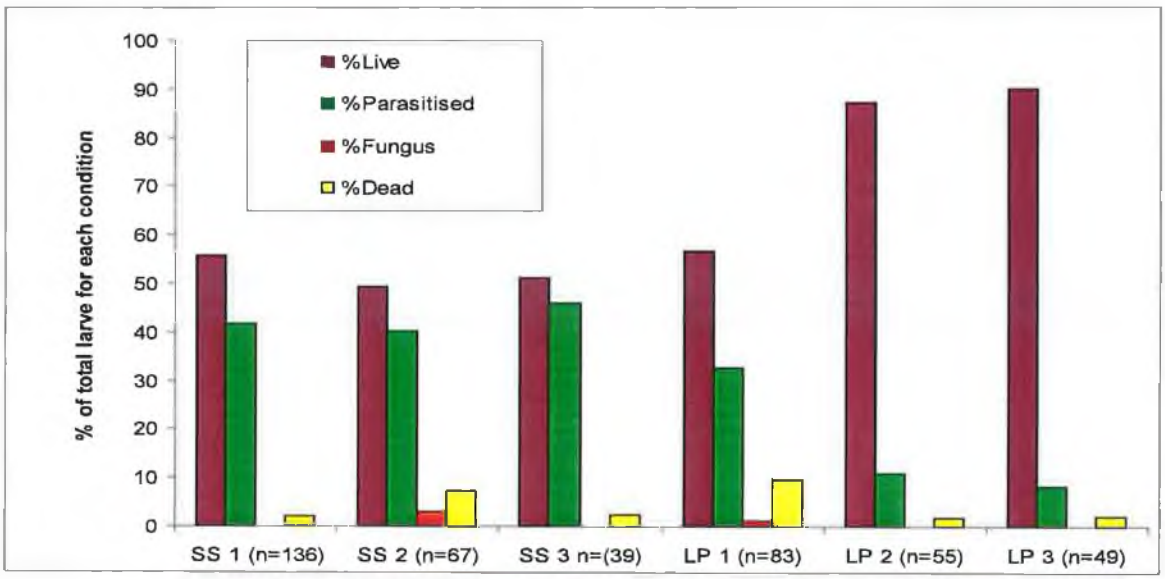

Figure 8.5. Condition of *H. abietis* in 3 LP and 3 SS logs, 16-20 days after introduction of *B. hylobii*.

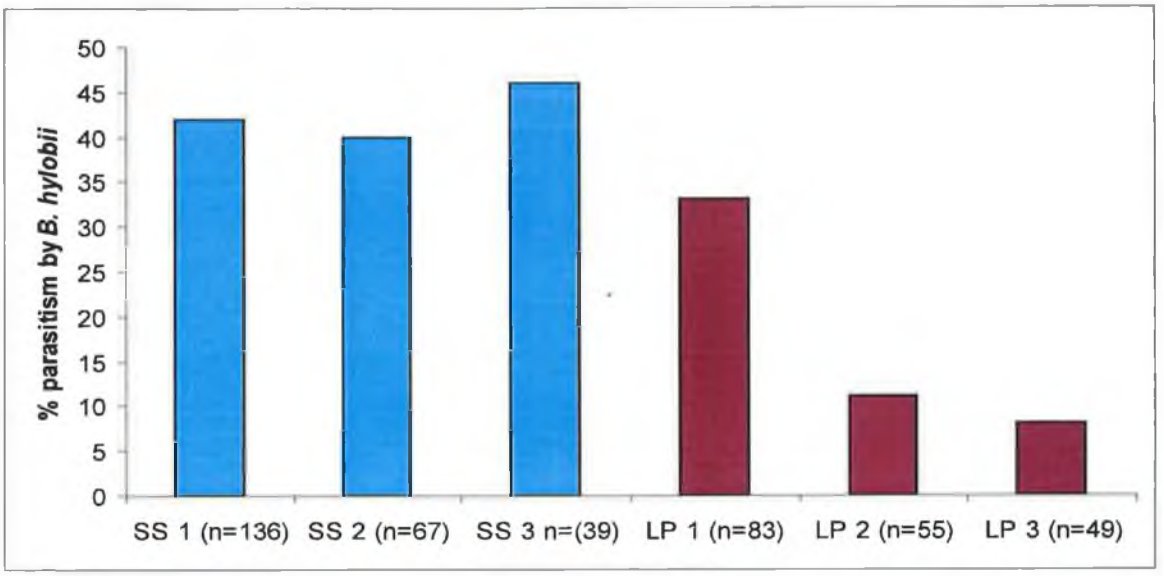

Figure 8.6. % Parasitism caused by *B. hylobii* in the 6 sampled logs (3SS and 3LP)

The frequency of *H. abietis* larvae, alive and parasitised was recorded against depth from the cut surface of the log. The location of the parasitised larvae in relation to the soil level was also recorded. It was noted that the majority of the parasitism occurred where the majority of the weevils were located which was to be expected but also, most of the parasitism occurred above the soil level even though there were hosts available below this level (Figures 8.7-8.12).

One other interesting observation when stripping the logs was that much of the parasitism was clumped within the logs i.e. weevils in close proximity to each other were often parasitised while other groups were not parasitised at all.

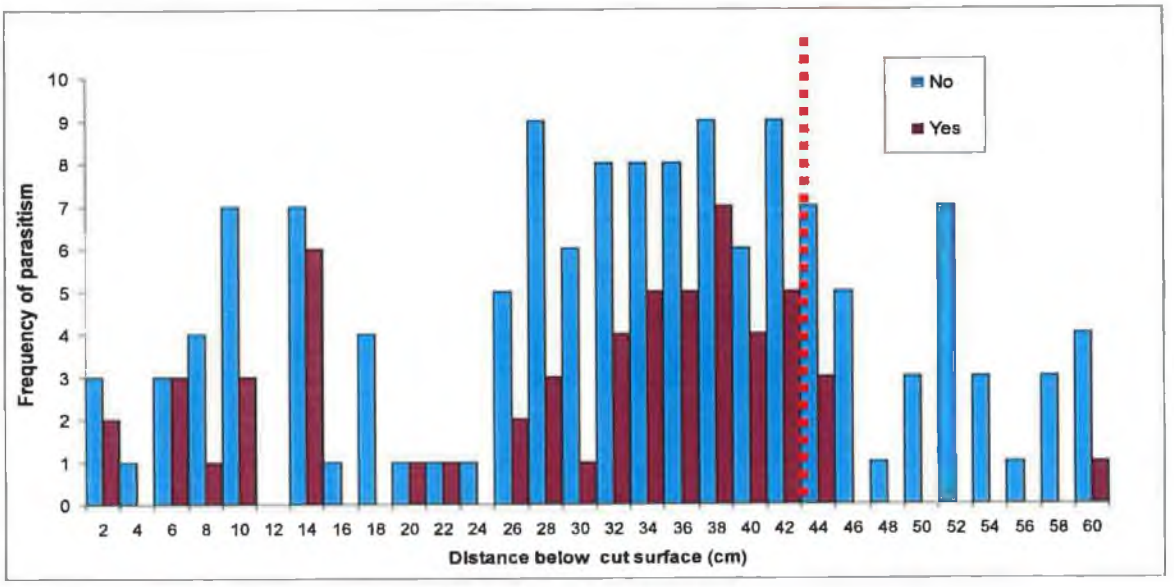

Figure 8.7. Frequency of parasitism in relation to distance from surface and soil depth in SS 1

'No' indicates the number of weevils which were not parasitised, while the 'Yes' indicates those which were parasitised by *B. hylobii*. The broken line indicates the level of the soil in each bin.

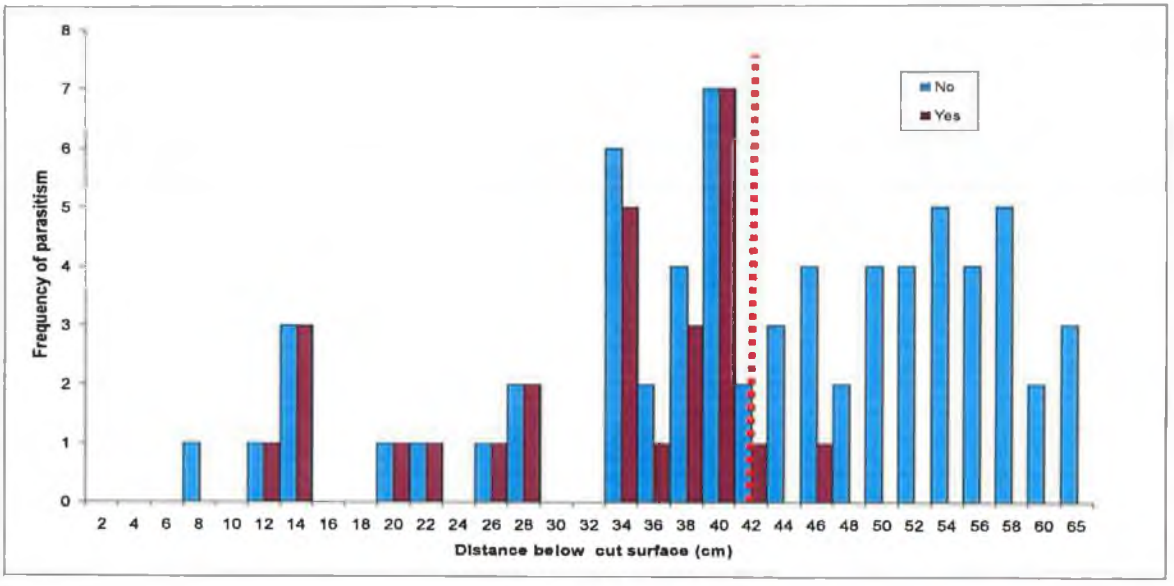

Figure 8.8. Frequency of parasitism in relation to distance from surface and soil depth in SS 2

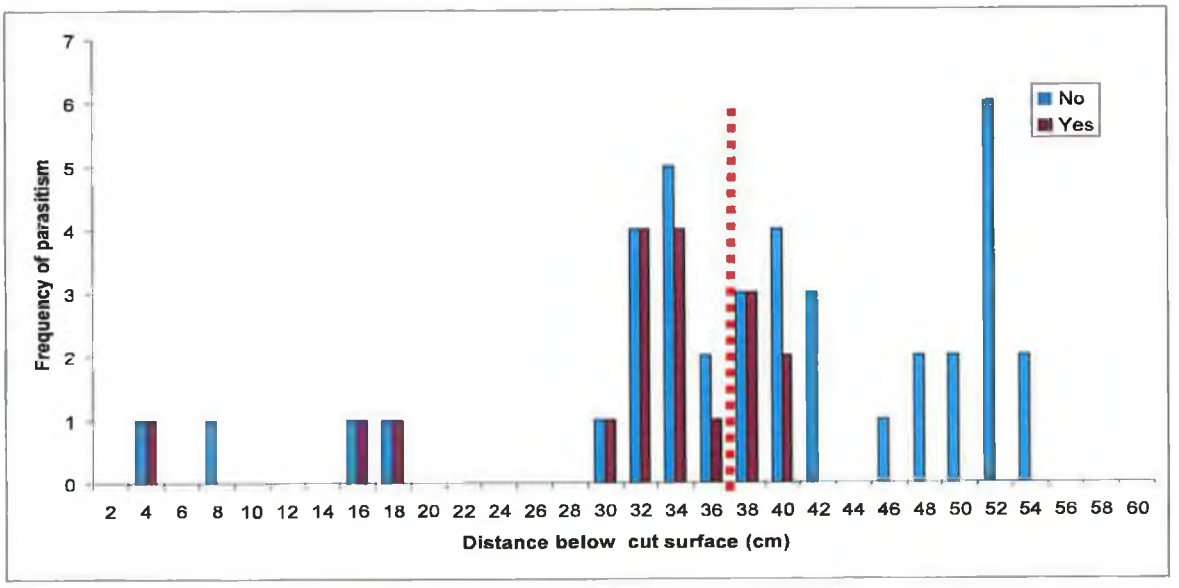

Figure 8.9. Frequency of parasitism in relation to distance from surface and soil depth in SS 3

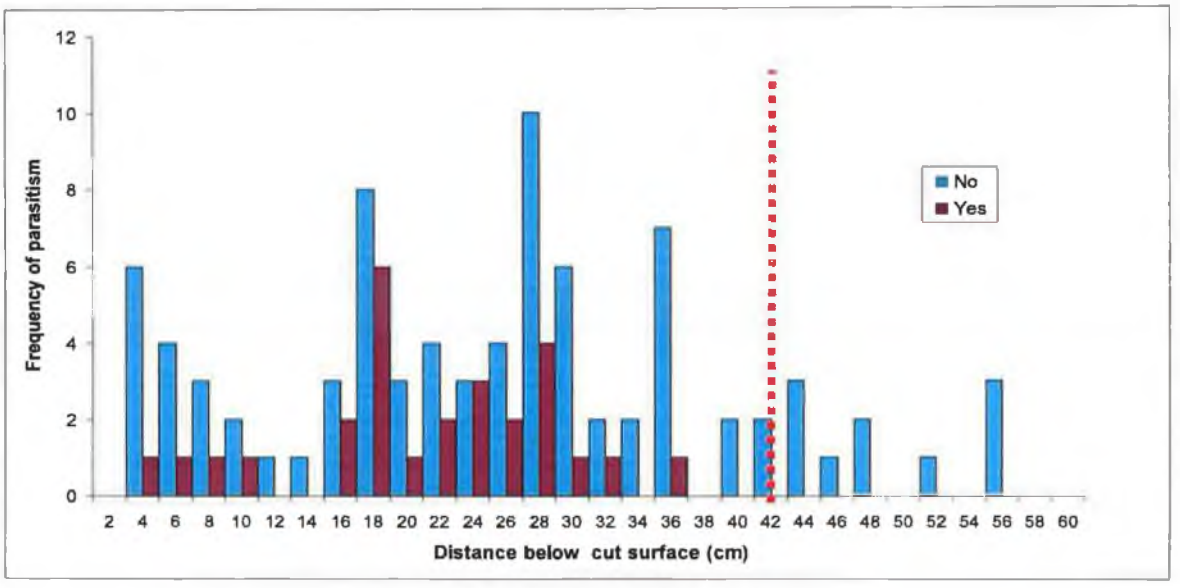

Figure 8.10. Frequency of parasitism in relation to distance from surface and soil depth in LP 1.

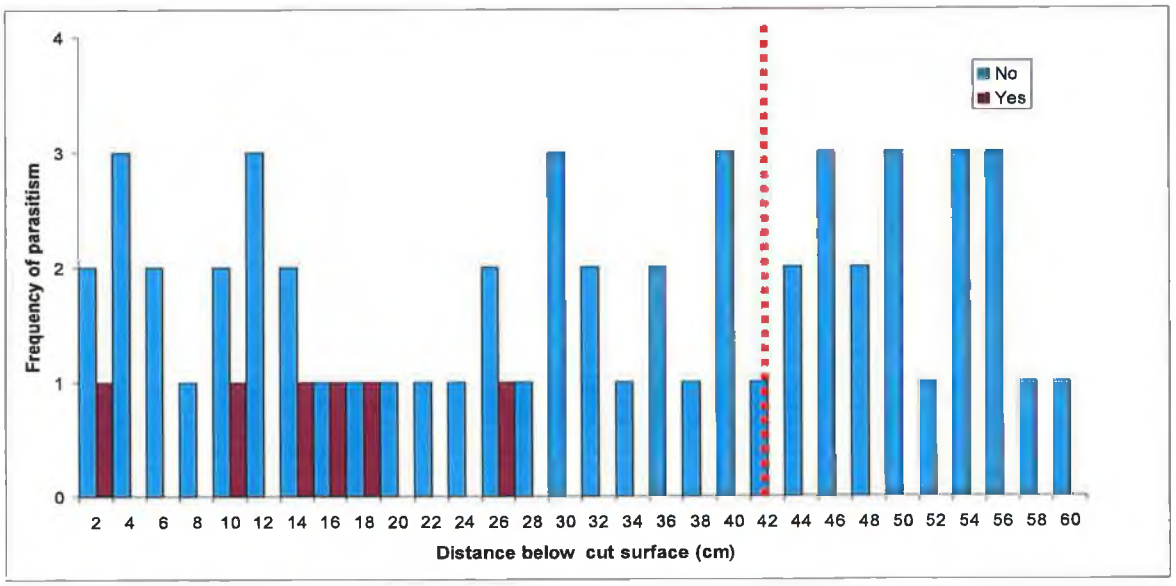

Figure 8.11. Frequency of parasitism in relation to distance from surface and soil depth in LP 2.

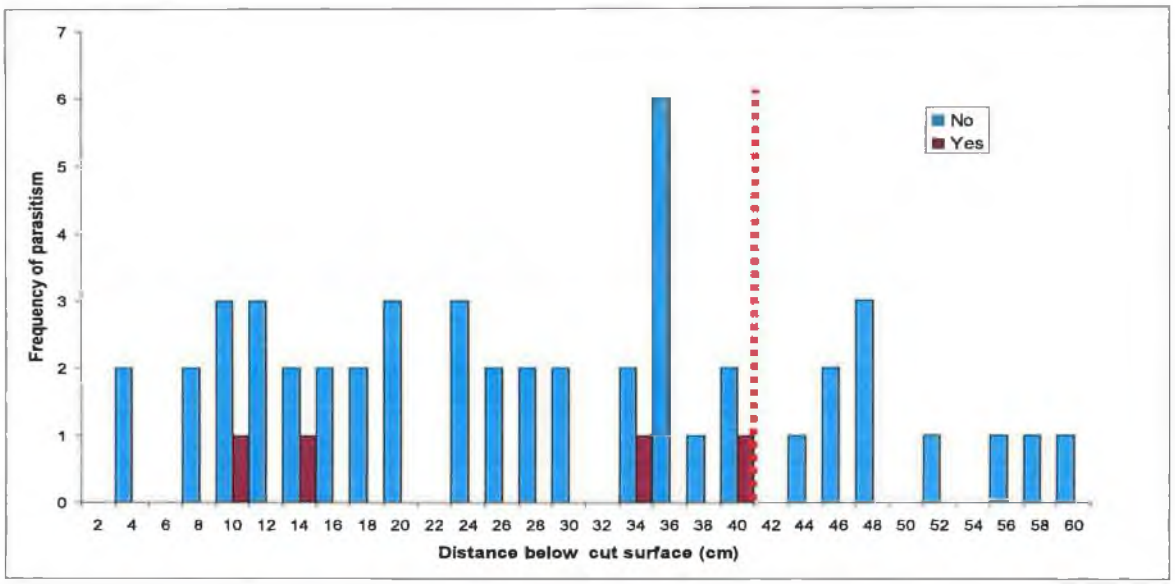

Figure 8.12. Frequency of parasitism in relation to distance from surface and soil depth in LP 3.

Total weevil numbers including the presence of empty chambers and parasitism by *B. hylobii* was recorded for the 20 'Lab infective units' it was also noted whether or not wasps had emerged from the cocoons (Table 8.2).

Table 8.2. Details of 'Lab Infective units'.

Label	Diameter	Total Weevils including empty chambers	Parasitised by bracon	% parasitism including empty chambers	Total number of cocoons	Empty cocoons	Full cocoons	Aver No. of cocoons per clutch
SSA	24	64	49	76.6	187	13	174	4
SSB	20	75	53	70.7	201	105	96	4
SSC	28	96	57	59.4	252	86	166	4
SSD	26	38	18	47.4	103	82	21	6
SSE	18	62	44	71.0	171	44	127	4
SSF	23	74	51	68.9	277	167	110	5
SSG	24	58	41	70.7	190	118	72	5
SSH	22.5	50	45	90.0	289	141	148	6
SSI	23	72	58	80.6	313	176	137	5
SSJ	23	65	37	56.9	246	111	135	7
Average	12	33	23	35	111	52	59	3
LPA	30	46	13	28.3	110	101	9	8
LPB	29	55	9	16.4	52	33	19	6
LPC	28	36	8	22.2	105	89	16	13
LPD	29	40	13	32.5	89	70	19	7
LPE	25	59	5	8.5	30	23	7	6
LPF	24	36	21	58.3	164	63	101	8
LPG	23	31	14	45.2	84	48	36	6
LPH	22	41	15	36.6	108	55	53	7
LPI	26	42	5	11.9	23	14	9	5
LPJ	23	40	9	22.5	35	14	21	4
Average	13	21	6	14	40	26	15	3

The mean percent parasitism in the stumps around the SS 'Lab infective units' and the LP 'Lab infective units' and those in the in the control area was 24.7%, 25.3% and 23% respectively on the SS site. The mean percent parasitism in the stumps around the SS 'Lab infective units' and the LP 'Lab infective units' and those in the control area was 33%, 50.6% and 43.8% respectively on the LP site. The 10m stumps had a mean percent parasitism of 27.8% and 36.9% on the SS site and LP site respectively. The SS and LP 'Lab infective units' on the SS site had a mean percent parasitism of 65% and 21.6% respectively with the corresponding values being 73.4% and 34.9% respectively on the LP site (Figure 8.13 and 8.14).

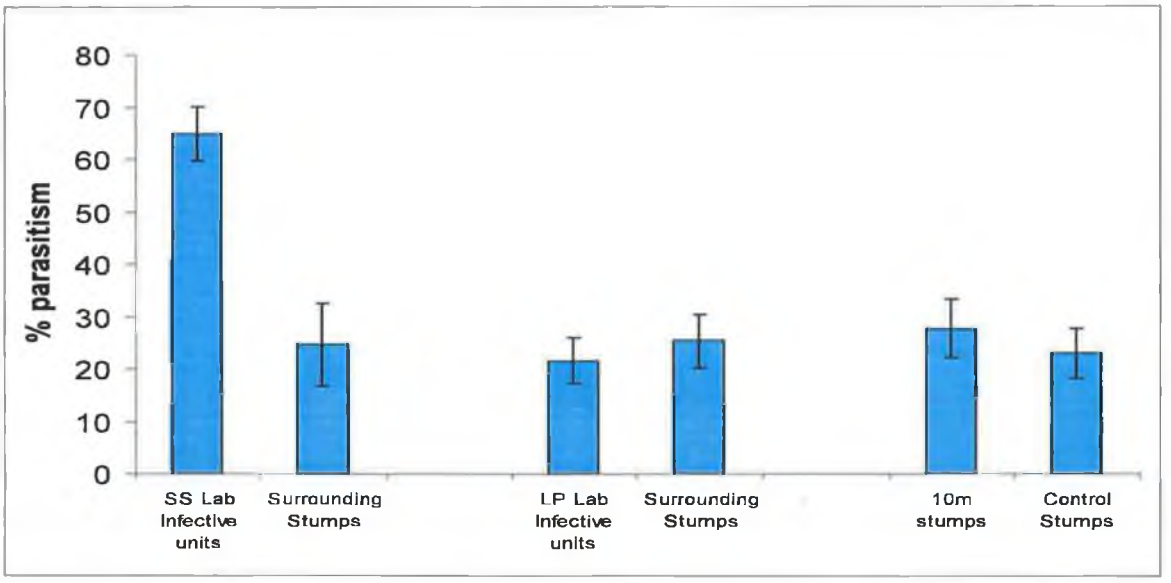

Figure 8.13. Average % Parasitism on SS site of the Lab infective units, the surrounding stumps and the control stumps.

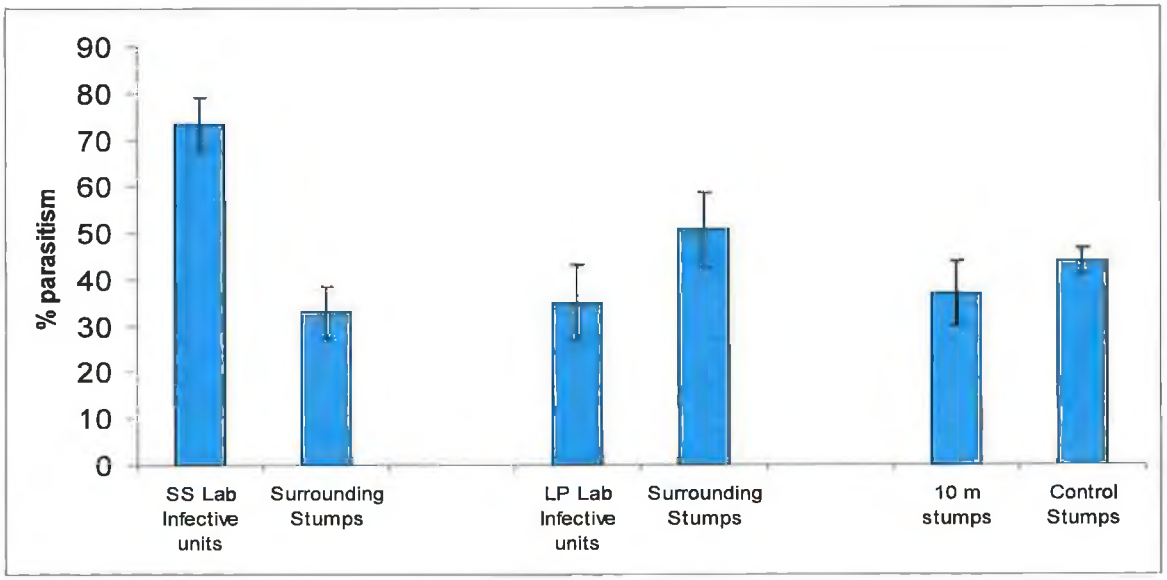

Figure 8.14. Average % Parasitism on LP site of the Lab infective units, the surrounding stumps and the control stumps.

Again these results indicate that the 'Lab infective units' had no effect on the rate of parasitism in the surrounding stumps on either the SS or LP site ANOVA ($F_{3,38}$=0.299, P=0.825) on the SS site and Kruskal-Wallis (P = 0.373, d.f. = 3, H = 3.12) on the LP site.

While stripping the 'Lab infective units,' it was noted that most logs had both empty and full cocoons. However, when the data were analysed there was no correlation between the number of empty cocoons in the 'Lab infective units' and the rate of parasitism in the surrounding stumps.

8.4 Field enhancement through inundative release

8.4.1 Introduction

The success of any bio-control inundative release program depends largely on a successful breeding program. A successful breeding program needs to produce large numbers of the control agent in a quick cost effective manner. Production of large numbers is made easier by the use of an artificial diet, on which the control agent can breed, as then the program is not reliant on the production of large numbers of hosts (Siddiqui and Dey 2002). However there is not presently an artificial diet available on which *B. hylobii* can breed, therefore a continuous supply of hosts (*H. abietis* larvae) are required to breed large numbers of *B. hylobii*. *Hylobius abietis* larvae can be gathered in two ways; they can be collected in the field from suitable sites or can be bred in the laboratory by allowing adults to lay eggs in conifer logs. Field collected larvae can be transferred to new logs to allow parasitism by *B. hylobii*. *Hylobius abietis* larvae can be bred relatively easily in conifer logs in the lab and the development time to a suitable stage for breeding *B. hylobii* can be decreased by elevating temperature. A problem that occurs is that the number of weevil larvae produced in logs by allowing adults weevils to lay eggs in them can be irregular. To regulate the numbers of *H. abietis* larvae available in a given log to a *B. hylobii* female(s), a set number of *H. abietis* larvae can be transferred from breeding logs to a new log, in the same way as field collected larvae, which is then made available to *B .hylobii* female(s), the method of doing this and a breeding protocol for *B. hylobii* is outlined below. In this case due to time constraints field collected larvae were used. The breeding technique used is loosely based on a method used in the Department of Entomology, University of California, to mass rear *Syngaster lepidus* and *Jarra phoracantha* (Hymenoptera:Braconidae), larval parasitoids of the phloem-colonizing longhorned beetles *Phoracantha semipunctata* and *P. recurva* (Coleoptera: Cerambycidae) (Millar *et al.* 2002).

Cocoons gathered from the breeding program were used in an inundative release of *B. hylobii* on 4 clearfell sites felled approximately 16 months previously. This was carried out during the second week of June 2007.

8.4.2 Material and methods

One thousand *H. abietis* larvae were collected from a Lodgepole pine site approximately 16-18 months post felling. Twenty Sitka spruce logs were cut approximately 50cm long by 20cm diameter. Forty eight larvae were introduced and held in each log by lifting a 2cm wide strip of bark and drilling a 10mm hole into the wood underneath approximately 2cm deep. Larvae were placed into the hole and covered with the bark strip, which was stapled down to hold each larva in place. This was done for each of 960 larvae. Each log was placed in a 90L plastic bin (breeding chamber (Figure 8.15) with some moss peat and covered with an organza mesh. Ten newly emerged female *B. hylobii* and a similar number of males were introduced into each bin and supplied with a 50:50 honey solution. Logs were held for 16 days at room temperature, at which time the stapled strip of bark was lifted and larvae assessed for parasitism. In most cases parasitism occurred in the hole in which the larva was originally placed or very close to where the larva had begun to feed (Figure 8.16).

Larvae were originally collected from an LP site as large numbers of *H. abietis* larvae were present on the site. They were transferred to SS logs as the bark is generally thinner and so would prove easier for the *B. hylobii* to locate and parasitise the *H. abietis* larvae.

Figure 8.15. *B. hylobii* feeding chamber.

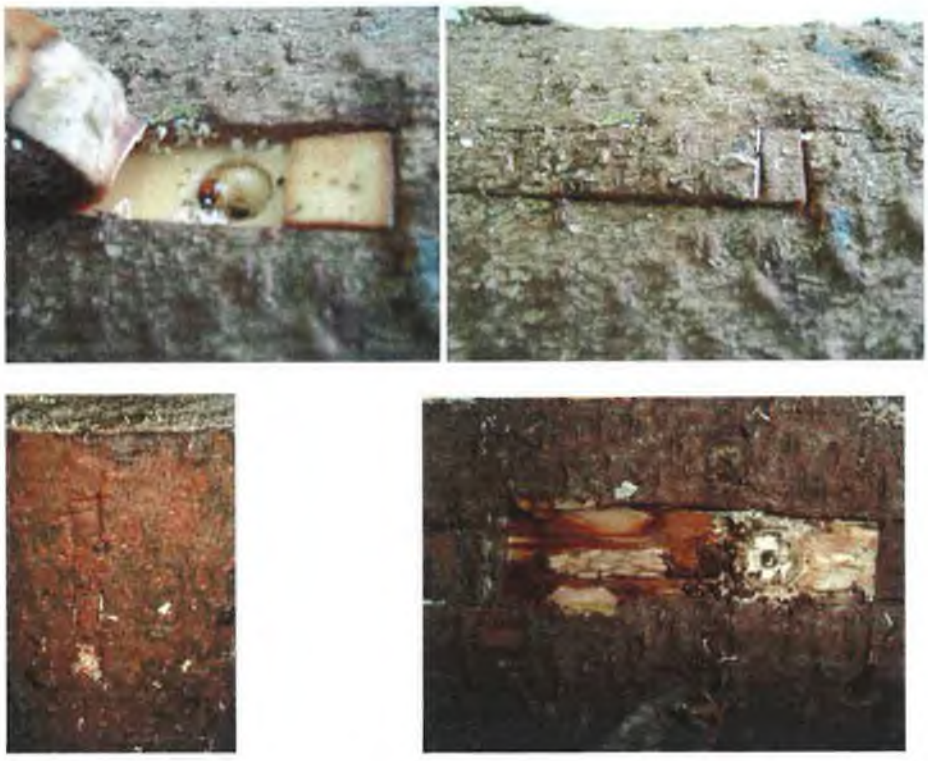

Figure 8.16. Breeding method developed for rearing large numbers of *B. hylobii*

Bracon hylobii cocoons produced in the breeding experiment were separated into 4 release chambers with 1200 in each. These were held in the laboratory until adults were seen to emerge (24 days after introduction of the *B. hylobii* in the breeding experiment). Containers were not transferred to the field until a number of adults had emerged to ensure that diapause would not be induced in the field. At that point (12-13 June) the containers were moved to 4 SS clearfell sites on peat that had been felled approximately 16 months previously. These sites had been assessed in the previous 2 weeks for the presence of *H. abietis* larvae. Stumps destructively sampled at that time on each site revealed that actively feeding *H. abietis* larvae were present, no *B. hylobii* were found. Approximately 6 stumps on each site were assessed.

In the field the containers protected the cocoons from predation while allowing the adults to emerge. Containers were held down by pegs and covered by a large bin lid to protect them from the elements (Figure 8.17).

Each release site and control area was assessed for percent parasitism during the week starting the 19th of November. The sites were not assessed until *B. hylobii*

emergence had ceased in the field; this time was gauged from numbers recorded in the emergence trap experiment. The 4 nearest stumps to the release point (in a North, South, East and West direction) were destructively sampled. A further 4 stumps in the same layout but 10m from the release point were also surveyed. An equal number of stumps in the same pattern were surveyed at the control point and compared with the treatment stumps.

Figure 8.17. Containers and covers used for field release of *Bracon hylobii*

8.4.3 Results

This proved a very successful and relatively easy way of rearing large number of *B. hylobii*. Over 5400 *B. hylobii* were produced. The outcomes of the breeding experiment are outlined in Table 8.3. Of the 960 larvae, originally transferred from the field to the SS logs in the lab, 619 or 64.4% were parasitised (Figure 8.18).

Table 8.3. Results of breeding experiment

Total *H. abietis* larvae introduced into the 20 SS logs	960
Total *H. abietis* larvae alive after 16 days	123
Total *H. abietis* larvae killed by fungus after 16 days	92
Total *H. abietis* pupae alive after 16 days	126
Total Parasitised *H. abietis* larvae after 16 days	619
Total *B. hylobii* cocoons recorded after 16 days	5449
Average *B. hylobii* cocoons produced per introduced *H. abietis* larvae	6
Average *B. hylobii* cocoons produced per parasitised *H. abietis* larvae	9

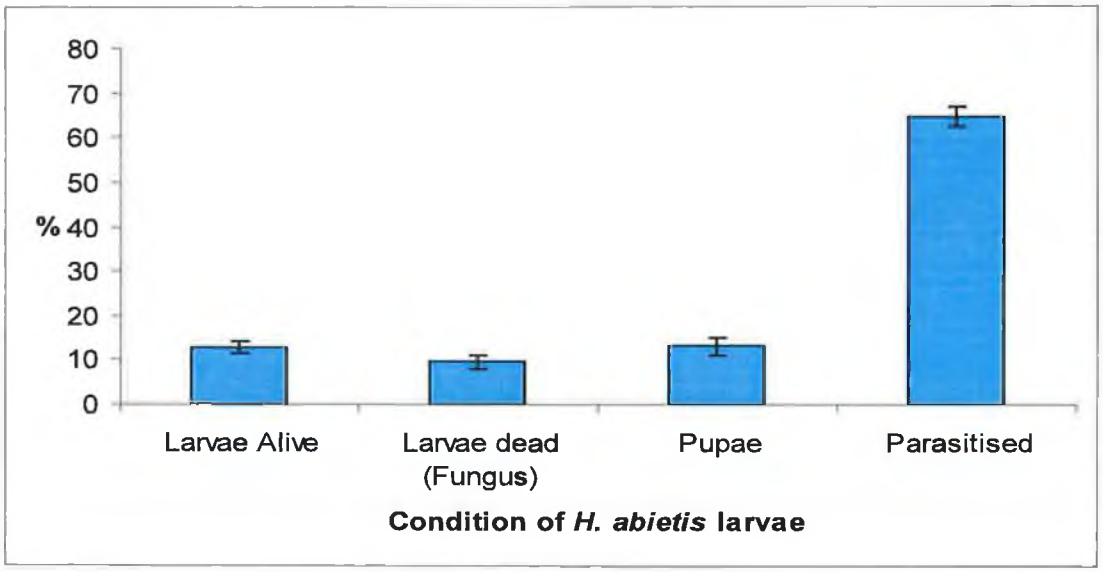

Figure 8.18. Condition of *Hylobius abietis* larvae (Mean ± SE) in breeding experiment 17 days after introduction of *Bracon hylobii* (N=960)

Mean percent parasitism rates of 46.4%, 33.3%, 22.8% and 2.1% were recorded in the treatments areas on each of the 4 sites respectively while the corresponding mean percentage rate on the control areas for the 4 sites were 34.4%, 35.5%, 10.5% and 1.9% respectively (Figure 8.19). However the differences proved not to be significant ANOVA ($F_{1,7}$=0.149, P=0.71079) after arcsine transformation.

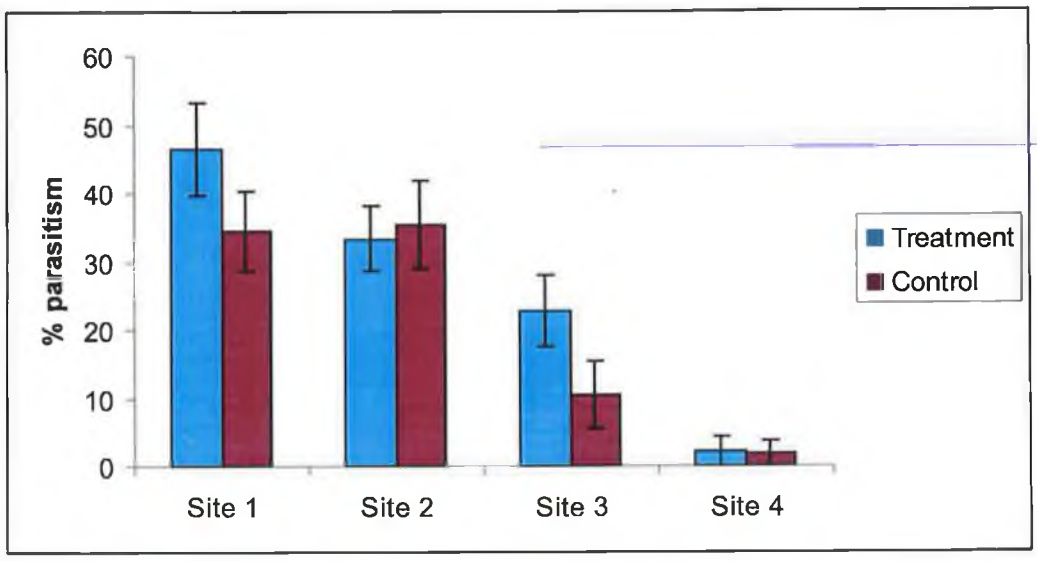

Figure 8.19. Percent parasitism (Mean ± SE) of *Hylobius abietis* by *Bracon hylobii* in treatment and control stumps on 4 Sitka spruce Sites (n = 8 stumps for each treatment and control)

8.5 Discussion

Attempts to enhance the rate of parasitism achieved by *B. hylobii* in the field through inoculation by transporting logs containing *B. hylobii* cocoons from one site to another or from the lab to field proved unsuccessful. The average percent parasitism in the stumps surrounding the 'Infective units' was very similar to that recorded in control stumps. This could be due to a range of factors including a lack of an adequate food resource on site (chapter 1.6.4) thus causing the wasp to disperse, the wasp entering diapause, the availability and accessibility of hosts, superparasitism or the presence of a hyperparasitoid.

Details from the sample of the 6 logs in the 'Lab infective units' trial indicated that Sitka spruce logs showed greater potential than Lodgepole pine logs for breeding *Bracon hylobii*. Breeding of *B. hylobii* in the bins was successful, with over 250 cocoons in some logs. Also the majority of the parasitism that occurred in the breeding chambers took place above the soil level even though hosts were available below this level. These results concur with results from previous experiments (chapter 7). The soil may act as both a physical barrier to the female wasp and or could in fact be acting as a barrier to the host finding cues used by the female. A study on scoliid wasps which parasitise soil-dwelling scarabaeid larvae found that the

wasp used chemical cues left by the larvae as they moved through the soil and was unable to detect larvae which had been artificially placed below the soil (Inoue and Endo 2008). As *H. abietis* larvae do not move through the soil, except at the very early stages of development, there are therefore no chemical cues left in the soil for female *B. hylobii* to detect. Therefore unless the wasp can physically penetrate the soil to reach the surface of the below ground portions of the stump it may not perceive the hosts below the soil surface. However in the field parasitism regularly takes place below soil level (Henry 1995; Henry and Day 2001; pers. ob.). It is this ability to move within openings in the soil and between the soil and the stump and even through larval galleries that enables *B. hylobii* to perceive, locate and attack hosts in parts of the stump below ground level. However the question stills remains as to how much time and energy females will spend locating less accessible hosts below the soil surface before dispersing?

In the lab *B. hylobii* cocoons were present in the logs within 17 days of the adult wasps being introduced. *Bracon hylobii* cocoons, larvae and eggs were present in the same log at the same time after 17 days. This indicates a prolonged oviposition period which could result in overlapping of generations.

In the lab, even with the removal of most of the soil from around the logs which gave the introduced wasps easy access to the majority of the weevil larvae in the logs, the highest rate of parasitism achieved was only 46%. This figure was achieved by the introduction of 5 adult females. When the result is compared to those for the experiment where increasing numbers of wasps were introduced (chapter 7) it correspond to a figure of 48% and 64% parasitism in SS and LP respectively when 5 females were introduced. This would seem to confirm that in lab conditions higher numbers of females are required to achieve higher rates of parasitism.

This method proved to be a successful way of producing large numbers of *B. hylobii*. From the results of previous experiments carried out (chapter 7) it is thought the rate of parasitism achieved could be increased by the addition of greater numbers of females to each breeding chamber. As mentioned the process could be simplified further by also producing/developing the *H. abietis* larvae in logs in the laboratory so

there would be no need for continuous collection of *H. abietis* larvae from the field. Some of the *H. abietis* larvae produced in the logs could be allowed to develop to adults so there would be no need to collect adults from the field, again reducing the amount of time and effort needed. Also some of the *B. hylobii* produced can be stored as cocoons and used again in the process when adults are needed. The most time consuming part of the process is creating the hole and bark strip in the new logs for the transferred larvae. Again this is done to regulate both the number of *H. abietis* larvae in each log and also the development stage of the larvae. This stage could be left out and the *B. hylobii* adults could be allowed access to the original logs in which the adult *H. abietis* had laid its eggs. These modifications would lead to a reasonably simple method off producing a continuous supply of both *H. abietis* and *B. hylobii* for both release and experimentation purposes in a similar way as Millar (2002) did in his mass rearing program. The advantages and disadvantages of not transferring a known number of weevils would have to be assessed based on time and labour constraints.

Although the rearing of *B. hylobii* proved successful the following inundative release of wasps onto sites failed to significantly raise the mean percent parasitism in the target area above background levels. Reasons for this are still not fully understood but again, as with the inoculation experiments the possible immediate dispersal of emergent wasp from the target area could be a major factor. Also, in the case of the inundative release, the very poor weather which occurred during the period almost from the date of release may have had a negative effect. The post emergence activity of the wasp in the field needs to be investigated further.

9 Artificial clearfell experiments

9.1 Artificial clearfell -1

9.1.1 Introduction

When assessing if the introduction of logs containing *B. hylobii* or the release of the parasitoid onto a clearfell site increased the rate of parasitism there are many confounding factors to take into consideration. One of these factors is the effect of the presence, or lack of, flowering plants. The importance of flowering plants to conserve or retain natural or introduced populations of a control agent has been discussed previously (chapter 1.6.4 and 1.8.5). In relation to *B. hylobii* previous experiments in this research program have indicated that newly emergent adults may put the need to feed above the need to attack hosts (chapter 5). A lack of an adequate food source was thought to be a one possible reason for the lack of success in the field enhancement experiments (chapter 8). In an attempt to minimise some of the confounding factors found on clearfell sites and to test if the addition of flowering plants affects the rate of parasitism by assisting in the retention of adults on site that may otherwise disperse to locate a food source, it was decided to set up an 'artificial clearfell site' into which logs containing *B. hylobii* cocoons were introduced.

9.1.2 Objective

The objective of this experiment was to test if adult *B. hylobii* could be retained on an 'artificial' reforestation site through the addition of flowering plants and if this would lead to increased rates of parasitism.

9.1.3 Materials and methods

During November 2005 adult weevils were introduced into 30 breeding chambers containing SS logs approximately 30cm in diameter by 60cm in length. These logs were used as the stumps in the artificial clearfell.

The artificial clearfell site was basically a green field site on a farm located near the town of Headford in Galway. There were no clearfells or second rotation plantations

close to the site. On the 5th of April 2006, 20 of the logs containing weevil larvae mentioned above were cut in half (2 x 30cm logs) and buried 15 cm into the ground on the green field site. Four treatment areas were created, each containing 8 logs/stumps at 2m x 2m spacing. A further 8 half logs were placed 10 metres away from the treatment areas. Two of the treatments areas contained supplementary planting of various flowering plants, which would be sources of food for the emerging *B. hylobii* (Figure 9.1 and 9.2) while the other two treatment areas were mowed regularly to keep them free of wild flowers. The treatment areas were spaced far enough apart so as not to affect each other. The remaining 8 full logs (2 had been stripped to assess the development stage of the larvae) were inoculated with *B. hylobii* on the 28th of March and moved to the site on the 11th of April. One of the 8 logs containing the *B. hylobii* cocoons ('Infective units') was placed in the centre of each set of 4 stumps (Figure 9.2).

Figure 9.1. Layout of stumps in first "artificial clearfell site"

Figure 9.2. Supplementary planting in first "artificial clearfell site"

The emergence of the wasps for the 'Infective units' was gauged by peeling back a small square of bark on one of the logs. This revealed a clutch of cocoons, which were assessed during the month of April to ascertain timing of emergence, and thus when the surrounding artificial stumps could be surveyed for percent parasitism. On the 1st of May, 15 adult female *B. hylobii* were also released into the centre of each treatment block. It was hoped this would augment the numbers emerging from the cocoons in the 'Infective units'. The surrounding stumps were assessed on the 16th of June

9.1.4 Results

Very poor rates of parasitism were achieved. Only 3 of the surrounding stumps contained parasitised larvae. This translated to 6.8%, 2.5% and 4.3% parasitism in each of the logs respectively. Of the 593 weevil larvae available in the surrounding stumps only 8 were parasitised amounting to 1.3% parasitism overall (Table 9.1).

Table 9.1. Results of first "artificial clearfell" experiment.

Each block contained 2 infective unit surrounded by 4 stumps, stumps E-L were the stumps placed 10m from Infective units.

Infective unit (IU)	Location	Flowers	Cocoons in IU	Empty cocoons in IU	*H. abietis* Larvae in surrounding 4 stumps	*H. abietis* Larvae Parasitised in surrounding 4 stumps	No. Cocoons	% parasitism
1	Block 1	N	91	18	63	0	0	0.0
2	Block 1	N	0	0	91	0	0	0.0
3	Block 2	Y	0	0	74	5	22	6.8
4	Block 2	Y	197	45	29	0	0	0.0
5	Block 3	N	21	8	25	0	0	0.0
6	Block 3	N	67	5	46	0	0	0.0
7	Block 4	Y	63	10	40	1	16	2.5
8	Block 4	Y	293	80	73	0	0	0.0

	10m Stumps	Nearest 'IU'	*H. abietis* Larvae in stump	*H. abietis* Larvae Parasitised in stump	No. Cocoons	% parasitism
	E	1,2	15	0	0	0.0
	F	3,4	47	2	14	4.3
	G	1,5	14	0	0	0.0
	H	2,6	12	0	0	0.0
	I	3,7	17	0	0	0.0
	J	4,8	8	0	0	0.0
	K	5,6	25	0	0	0.0
	L	7,8	14	0	0	0.0
Totals			**593**	**8**	**52**	**1.3**

9.2 Artificial clearfell -2

9.2.1 Introduction

When considering reasons for the low rates of parasitism achieved in the first artificial clearfell experiment it was thought that the timing of the experiment may have been too early in the season, as many of the cocoons present in the introduced logs did not eclose as they may have entered diapause. Results from the emergence experiment (chapter 3) which were being analysed at the time also indicated that emergence in the field does not occur until late April/early May, peaking in June and July. In an attempt to minimise the effect of temperature induced diapause a similar artificial clearfell experiment was set up on the 25th of July 2006, again on a green field site.

9.2.2 Objective

The objective of this experiment was to test rates of parasitism by released *B. hylobii* on an 'artificial' reforestation site and to test if parasitism rates varied in different Hylobius food substrate. Again this experiment was carried out in an attempt to minimise the confounding factors found on a 'real' reforestation site.

9.2.3 Materials and methods

This experiment consisted of 4 blocks of 12 logs approximately 20m apart. Each block consisted of 6 LP and 6 SS spaced alternatively 2m apart. Additionally 3 logs were laid out between the blocks (Figure 9.3). All the logs were partially buried at a slant and partially covered with moss peat. All logs had previously been inoculated with *H. abietis* in the lab, again using the breeding chamber method.

Figure 9.3. Layout of logs (Alternative SS and LP) in 2nd artificial clearfell experiment

Field collected cocoons were allowed to emerge and mate in chambers in the lab. When sufficient females had emerged and mated (approximately 5 days later) 4 groups of 30 females were separated from the population. One of these groups was released in the centre of each block in the artificial clearfell on the 27th of July. Approximately 30-40 males were also released on the site. Cotton wool soaked in 50% honey solution was also placed in the centre of each block as a source of food for the released wasps. On the 17th of August, 21 days after the wasps were released all the logs were brought back to the laboratory and assessed for percent parasitism.

9.2.4 Results

As with the previous experiment the percent parasitism rates were quite low. Of the 51 logs in the experiment only 6 contained parasitised weevils. The rates achieved in these logs were, 10.7%, 6.5%, 2%, 5.3%, 9.1% and 14.3% respectively. While a total of 1261 *H. abietis* larvae were found in the logs only 14 were parasitised, this amounted to an overall parasitism rate of 1.1% (Table 9.2 and Figure 9.4).

Table 9.2. Results for 2nd "artificial clearfell" experiment.

Total *H. abietis*	Total *H. abietis* larvae	Total parasitism	% parasitism of all *H. abietis*	% parasitism of *H. abietis* Larvae
1774	1261	14	0.8	1.1

Stump No.	species	Location	Total *H. abietis*	Total *H. abietis* larvae	Parasitised *H. abietis* larvae	% parasitism of all *H. abietis*	% parasitism of *H. abietis* Larvae
5	SS	Block 1	43	28	3	7.0	10.7
11	SS	Block 1	52	46	3	5.8	6.5
18	SS	Block 2	57	49	1	1.8	2.0
25	SS	Block 3	38	38	2	5.3	5.3
29	SS	Block 3	33	22	2	6.1	9.1
47	LP	Block 4	32	21	3	9.4	14.3

	22(SS)	23(LP)	24(SS)		34(LP)	35(SS)	36(LP)	
	19(LP)	20(SS)	21(LP)		31(SS)	32(LP)	33(SS)	
	16(SS)	17(LP)	**18(SS)**		28(LP)	**29(SS)**	30(LP)	
	13(LP)	14(SS)	15(LP)		**25(SS)**	26(LP)	27(SS)	
49(SS)				50(LP)				51(SS)
	10(LP)	**11(SS)**	12(LP)		46(SS)	**47(LP)**	48(SS)	
	7(SS)	8(LP)	9(SS)		43(LP)	44(SS)	45(LP)	
	4(LP)	**5(SS)**	6(LP)		40(SS)	41(LP)	42(SS)	
	1(SS)	2(LP)	3(SS)		37(LP)	38(SS)	39(LP)	

Figure 9.4. Numbering and layout of artificial logs in 2nd "artificial clearfell" experiment

The highlighted numbers are the logs in which parasitism occurred.

A couple of interesting points of note were that of the 6 logs containing parasitised weevils 5 were SS logs. However the highest individual rate of parasitism was achieved in the LP log. As the number of logs containing parasitised larvae was so low it is not possible to say if the released wasps preferred SS to LP, however in the lab SS was consistently found to be better for breeding *B. hylobii* than LP, possibly because of the thinner bark. One other point of note was that the *B. hylobii* found on the weevils were still in the very early stages of development.

9.3 Discussion

As mentioned the reasons for this very low rate of parasitism achieved in the first experiment may be partially explained by the fact that when the 'Infective units' were assessed only 166 of the total 732 cocoons found had adult exit holes.. This meant that 77% of the introduced cocoons either entered diapause or the larvae/pupae inside died. The relatively low total number of cocoons found in the combined 8 'Infective units' was also surprising as previously in the laboratory close to 300 cocoons were often found in a single log. However this low emergence should have been somewhat mitigated by the release of adults directly into each treatment. As a consequence of the low parasitism rate it was not possible to test the effect of the addition of flowering plants.

In the second experiment the early development stage of the *B. hylobii* larvae found would suggest that the released wasp stayed on site but were slow to parasitise or that they may have left the site and returned later. Whether parasitoids that leave a patch in search of food will return to the original target area is unknown (Lewis *et al.* 1998). There is also the possibility that the released wasps left the site and another population of *B. hylobii* found the site, however due to the isolated nature of the site in relation to other clearfells this is not likely to have happened. Taking the development stage of the *B. hylobii* found it could be possible that if the logs were left on the site for longer more parasitism could have occurred. The reason for not leaving the logs on site for longer was not to allow a second generation of *B. hylobii* to emerge from the logs, as it was the effect of the released wasps that was being studied. However it would be of interest to study the cumulative effect of the release over the entire season/year.

10 Hyperparasite

10.1 Introduction

The presence of a hyper-parasite will negatively effect and limit a parasitoid as a successful biological control (Luck *et al.* 1981; Holler *et al.* 1993). No hyperparasites of *B. hylobii* have been recorded to date (Munro 1914; Henry 1995). However if was felt that hyper-parasitism may explain why a specific parasitoid is not as successful in controlling populations when compared to other species specific Braconidae.

During the period of research of this project no obvious morphological differences in wing venation were recorded in the *B. hylobii* emerging from any of the breeding experiments conducted or from those emerging from *B. hylobii* cocoons collected from the field. However during identification of the adult *B. hylobii* captured in the emergence traps it was noted that there was a great variance in the size and in the number of antennal segments in what, based on their wing venation appeared to be *B. hylobii* wasps. As with parasitoids in general, the size difference is not of great significance as this may merely be a result of poor host quality or size (King 1989; Henry 1995; Thompson and Hagen 1999). However the difference in the number of antennal segments needs to be investigated further. This was done through DNA analysis. DNA analysis would also rule out the presence of any cryptic hyperparasitoids.

10.2 Objective

The objective of this experiment was to test if *B. Hylobii* with different physical features such as size and number of antennal segments were a possible hyper parasite. A hyperparasite could be a factor in the low rates of parasitism achieved naturally?

10.3 Materials and methods

Approximately 100 *B. hylobii* cocoons collected from the field and stored at 5-6°C to maintain diapause. These were then divided into 10 groups of 10, in small containers and placed in an incubator at 20°C. After approximately 7-8 days all of the adults

which had emerged were transferred into vials of 70% alcohol. The venation of each of the adults was later examined and their antennal segments were counted. Whereas no discernible differences in wing venation were noted, there were differences in the numbers of antennal segments counted. As well as variation between wasps there was also variance within the two antennae of individual wasps. In total 8 specimens were selected for DNA analysis. These included a female with 25 segments in both antennae a female with 26 and 27 segments on either antennae, a female with 27 and 28 segments on either antennae and males with 27, 28, 29 and 30 segments on both antennae. It was hoped that any molecular differences would be identified in these samples and if so more samples could be tested.

The DNA analysis was carried out by Karl Magnacca, Postdoctoral Researcher, Department of Zoology, Trinity College, Dublin 2, Ireland. His extraction and sequencing methods are outlined below.

"DNA was extracted from 12 specimens representing the range of antennal segment variation. Total DNA was extracted using the DNeasy Blood & Tissue extraction kit (Qiagen Inc.), following the manufacturer's protocol. DNA was taken from the mid and hind right legs of each specimen, except for one [extraction 1, with 25 antennal segments] which was done by soaking the body in the lysis solution.

The "standard" barcoding fragment of cytochrome oxidase I was amplified using a version of the commonly-used primer LCO (5'–TATCAACCAATCATAAAGATATTGG–3') (Folmer *et al.* 1994), modified for use in Hymenoptera, with a shortened version of "Nancy" (5'–CCCGGTAAAATTAAAATATAAAC–3') (Simon *et al.* 1994) as the reverse. These would be called C1-J-1514 and C1-N-2194 under the Simon et al. (1994) naming scheme. PCR was run using standard Taq (Invitrogen Corp.) with the following program: a starting denaturation at 94° for 180 seconds, followed by 35 cycles of 94° for 30 s, 48° for 45 s, and 72° for 60 s, concluding with a final extension at 72° for 240 s.

PCR products were sequenced with an ABI 3130xl capillary automated sequencer (Applied Biosystems Inc.) in the School of Natural Sciences, Trinity College. The PCR primers were used for sequencing. All were sequenced from both directions,

although sequences were of high enough quality that only one direction was necessary. Chromatograms were edited using FinchTV (Geospiza Inc.).

Four of the extractions (3, 6, 7, and 9) amplified extremely weakly and were not sequenced; the remaining eight produced identical sequences. The sequencing failed in the reverse direction for one (12), but the forward sequence was extremely clean."

10.4 Results

Of the 8 specimens sequenced there was a 100% match across the range on antennal segments.

The sequences of the 8 specimens along with another Braconid wasp sequence for comparison are given in plain text format below (the number preceding the F/M indicates the number of antennal segments present and F or M indicates sex of the wasp).

Bracon_sp ----------

GGTAGGTTTATCAATAAGTATAATTATTCGTTTAGAATTAGGATCTCCTGGGAGATTATTAGGTAATG
ACCAAATTTATAATAGTATAGTTACTTCACATGCTTTTATTATAATTTTTTTTATAGTTATACCAATT
ATAATTGGTGGATTTGGGAATTGATTATTGCCGTTAATATTAGGGGCTCCTGATATAGCTTTTCCTCG
AATGAATAATATGAGATTTTGATTATTAGTTCCTTCAATTTCTTTATTGATATTAAGAGGTTTTATAA
ATATTGGAGTAGGTACAGGTTGAACAGCTTATCCTCCTTTATCTTCTACAATAGGACATGGAGGTACA
TGTGTTGATTTAGCTATTTTTTCTTTACATTTAGCTGGTATTTCTTCAATTATAGGTTCAATTAATTT
TATTACAACAATTTTAAATATACGTTTAATATCTTTAAAATTAGATCAGTTATCTCTATTTAGTTGAT
CAGTTTTTATTACTACAATTTTATTATTATTATCATTACCTGTTTTAGCTGGGGCAATTACTATATTA
TTAACTGATCGTAATTTAAATACTACTTTTTTTGATTTTTCGGGGGGGGGGGATCCAGT---------
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Brahyl_MM001_25F

TGTATTATATTTTTTATTTGGAATATGATCAGGTATATTAGGTTTATCAATAAGATTAATTATTCGTT
TAGAATTAGGAATCCCAGGAAGTTTATTAGGAAATGATCAAATTTATAATAGTATAGTGACAGCTCAT
GCTTTAATTATAATTTTCTTTATAGTTATACCTGTAATAGTTGGTGGATTTGGAAATTGATTATTACC
TCTAATATTAGGATCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGATTTTGATTAATTATTC
CATCATTAATTTTATTATTAATAAGAAGAATTTTAAATGTTGGGGTAGGAACTGGTTGAACAATTTAT
CCTCCTTTATCATCTTCATTAGGACATAGAGGAATATCTGTTGATTTAGCTATTTTTTCTTTACATAT
TGCTGGTATCTCCTCAATTTTGGGAGCAATTAATTTTATTACAACTATTTTTAATATACATTTATTTA
TTTTAAAATTAGATCAATTAACTTTATTAATTTGGTCAATTTTTATTACAGCTATATTATTATTGTTA
TCTTTACCAGTATTAGCAGGTGCTATTACAATATTATTAACTGATCGAAATTTAAATACATCTTTTTT
TGATTTTTCTGGAGGTGGAGATCCAATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCTGAA

Brahyl_MM002_2627F

TGTATTATATTTTTTATTTGGAATATGATCAGGTATATTAGGTTTATCAATAAGATTAATTATTCGTT
TAGAATTAGGAATCCCAGGAAGTTTATTAGGAAATGATCAAATTTATAATAGTATAGTGACAGCTCAT
GCTTTAATTATAATTTTCTTTATAGTTATACCTGTAATAGTTGGTGGATTTGGAAATTGATTATTACC
TCTAATATTAGGATCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGATTTTGATTAATTATTC
CATCATTAATTTTATTATTAATAAGAAGAATTTTAAATGTTGGGGTAGGAACTGGTTGAACAATTTAT
CCTCCTTTATCATCTTCATTAGGACATAGAGGAATATCTGTTGATTTAGCTATTTTTTCTTTACATAT
TGCTGGTATCTCCTCAATTTTGGGAGCAATTAATTTTATTACAACTATTTTTAATATACATTTATTTA
TTTTAAAATTAGATCAATTAACTTTATTAATTTGGTCAATTTTTATTACAGCTATATTATTATTGTTA
TCTTTACCAGTATTAGCAGGTGCTATTACAATATTATTAACTGATCGAAATTTAAATACATCTTTTTT
TGATTTTTCTGGAGGTGGAGATCCAATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCTGAA

Brahyl_MM004_27M

TGTATTATATTTTTTATTTGGAATATGATCAGGTATATTAGGTTTATCAATAAGATTAATTATTCGTT
TAGAATTAGGAATCCCAGGAAGTTTATTAGGAAATGATCAAATTTATAATAGTATAGTGACAGCTCAT
GCTTTAATTATAATTTTCTTTATAGTTATACCTGTAATAGTTGGTGGATTTGGAAATTGATTATTACC
TCTAATATTAGGATCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGATTTTGATTAATTATTC
CATCATTAATTTTATTATTAATAAGAAGAATTTTAAATGTTGGGGTAGGAACTGGTTGAACAATTTAT
CCTCCTTTATCATCTTCATTAGGACATAGAGGAATATCTGTTGATTTAGCTATTTTTTCTTTACATAT
TGCTGGTATCTCCTCAATTTTGGGAGCAATTAATTTTATTACAACTATTTTTAATATACATTTATTTA
TTTTAAAATTAGATCAATTAACTTTATTAATTTGGTCAATTTTTATTACAGCTATATTATTATTGTTA
TCTTTACCAGTATTAGCAGGTGCTATTACAATATTATTAACTGATCGAAATTTAAATACATCTTTTTT
TGATTTTTCTGGAGGTGGAGATCCAATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCTGAA

Brahyl_MM005_2728F

TGTATTATATTTTTTATTTGGAATATGATCAGGTATATTAGGTTTATCAATAAGATTAATTATTCGTT
TAGAATTAGGAATCCCAGGAAGTTTATTAGGAAATGATCAAATTTATAATAGTATAGTGACAGCTCAT
GCTTTAATTATAATTTTCTTTATAGTTATACCTGTAATAGTTGGTGGATTTGGAAATTGATTATTACC
TCTAATATTAGGATCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGATTTTGATTAATTATTC
CATCATTAATTTTATTATTAATAAGAAGAATTTTAAATGTTGGGGTAGGAACTGGTTGAACAATTTAT
CCTCCTTTATCATCTTCATTAGGACATAGAGGAATATCTGTTGATTTAGCTATTTTTTCTTTACATAT
TGCTGGTATCTCCTCAATTTTGGGAGCAATTAATTTTATTACAACTATTTTTAATATACATTTATTTA
TTTTAAAATTAGATCAATTAACTTTATTAATTTGGTCAATTTTTATTACAGCTATATTATTATTGTTA
TCTTTACCAGTATTAGCAGGTGCTATTACAATATTATTAACTGATCGAAATTTAAATACATCTTTTTT
TGATTTTTCTGGAGGTGGAGATCCAATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCTGAA

Brahyl_MM008_28M

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GCTTTAATTATAATTTTCTTTATAGTTATACCTGTAATAGTTGGTGGATTTGGAAATTGATTATTACC
TCTAATATTAGGATCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGATTTTGATTAATTATTC
CATCATTAATTTTATTATTAATAAGAAGAATTTTAAATGTTGGGGTAGGAACTGGTTGAACAATTTAT
CCTCCTTTATCATCTTCATTAGGACATAGAGGAATATCTGTTGATTTAGCTATTTTTTCTTTACATAT

TGCTGGTATCTCCTCAATTTTGGGAGCAATTAATTTTATTACAACTATTTTTAATATACATTTATTTA

TTTTAAAATTAGATCAATTAACTTTATTAATTTGGTCAATTTTTATTACAGCTATATTATTATTGTTA

TCTTTACCAGTATTAGCAGGTGCTATTACAATATTATTAACTGATCGAAATTTAAATACATCTTTTTT

TGATTTTTCTGGAGGTGGAGATCCAATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCTGAA

Brahyl_MM010_29M

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GCTTTAATTATAATTTTCTTTATAGTTATACCTGTAATAGTTGGTGGATTTGGAAATTGATTATTACC

TCTAATATTAGGATCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGATTTTGATTAATTATTC

CATCATTAATTTTATTATTAATAAGAAGAATTTTAAATGTTGGGGTAGGAACTGGTTGAACAATTTAT

CCTCCTTTATCATCTTCATTAGGACATAGAGGAATATCTGTTGATTTAGCTATTTTTTCTTTACATAT

TGCTGGTATCTCCTCAATTTTGGGAGCAATTAATTTTATTACAACTATTTTTAATATACATTTATTTA

TTTTAAAATTAGATCAATTAACTTTATTAATTTGGTCAATTTTTATTACAGCTATATTATTATTGTTA

TCTTTACCAGTATTAGCAGGTGCTATTACAATATTATTAACTGATCGAAATTTAAATACATCTTTTTT

TGATTTTTCTGGAGGTGGAGATCCAATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCTGAA

Brahyl_MM011_29M

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GCTTTAATTATAATTTTCTTTATAGTTATACCTGTAATAGTTGGTGGATTTGGAAATTGATTATTACC

TCTAATATTAGGATCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGATTTTGATTAATTATTC

CATCATTAATTTTATTATTAATAAGAAGAATTTTAAATGTTGGGGTAGGAACTGGTTGAACAATTTAT

CCTCCTTTATCATCTTCATTAGGACATAGAGGAATATCTGTTGATTTAGCTATTTTTTCTTTACATAT

TGCTGGTATCTCCTCAATTTTGGGAGCAATTAATTTTATTACAACTATTTTTAATATACATTTATTTA

TTTTAAAATTAGATCAATTAACTTTATTAATTTGGTCAATTTTTATTACAGCTATATTATTATTGTTA

TCTTTACCAGTATTAGCAGGTGCTATTACAATATTATTAACTGATCGAAATTTAAATACATCTTTTTT

TGATTTTTCTGGAGGTGGAGATCCAATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCTGAA

Brahyl_MM012_30M ----------

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ATTAGGAAATGATCAAATTTATAATAGTATAGTGACAGCTCATGCTTTAATTATAATTTTCTTTATAG

TTATACCTGTAATAGTTGGTGGATTTGGAAATTGATTATTACCTCTAATATTAGGATCTCCTGATATA

GCTTTCCCTCGTATAAATAATATAAGATTTTGATTAATTATTCCATCATTAATTTTATTATTAATAAG

AAGAATTTTAAATGTTGGGGTAGGAACTGGTTGAACAATTTATCCTCCTTTATCATCTTCATTAGGAC

ATAGAGGAATATCTGTTGATTTAGCTATTTTTTCTTTACATATTGCTGGTATCTCCTCAATTTTGGGA

GCAATTAATTTTATTACAACTATTTTTAATATACATTTATTTATTTTAAAATTAGATCAATTAACTTT

ATTAATTTGGTCAATTTTTATTACAGCTATATTATTATTGTTATCTTTACCAGTATTAGCAGGTGCTA

TTACAATATTATTAACTGATCGAAATTTAAATACATCTTTTTTTGATTTTTCTGGAGGTGGAGATCCA

ATTTTATTTCAACATTTATTTTGATTTTTTGGTCATCCTGAA

10.5 Discussion

These results would indicate that variable antennal segment number is due to natural variation within the species. However this alone is a useful point for future descriptions of *B. hylobii*. The description of *B. hylobii* by Ratzeburg, given in Munro (1914) suggests that the female has 31 antennal segments. These results prove this is not always the case and should be considered in future identifications of *B. hylobii*. The small size of the sample should also be considered before the possibility of a cryptic-hyperparasite is totally rejected.

11 Discussion and General Conclusions

Results from emergence experiments (chapter 3) have shown that there are 2 major peaks of emergence by *B. hylobii*. The first peak (consisting largely of the overwintered population) occurs in late spring and the second approximately 8-9 weeks later during the summer. Smaller peaks were recorded up to the end of November. This indicates 2 or maybe 3 generations per year. Data from the emergence traps indicate that even though there are peaks of emergence, *B. hylobii* is active on site at lower levels around these peaks. This long period of activity (May to November) should improve its potential as a control agent in so far as it means there is a large window of opportunity to find and parasitise hosts. It may also aid the wasp in dealing with overlapping of generations of the weevil in that there will be adult wasps on site outside the times in which the primary weevil generation develops. Results from both the emergence and artificial clearfell experiments coupled with Henry's (1995) and Henry and Day's (2000) research yielded useful information on the best timing of a release program so as to coincide with either natural peaks or troughs in local natural population. The results also indicate the best period for a release in relation to climatic factors and season. Until now little was known about the timing of both first and peak emergences in the field of *B. hylobii*. The information gathered in this research may be useful if *B. hylobii* was to be used in conjunction with other control measures such as nematodes in an integrated pest management strategy of *H. abietis*. Applications of the nematodes could be targeted at times to limit exposure of susceptible stages of *B. hylobii* to parasitism by the nematodes.

Breeding of *B. hylobii* in chambers in the lab has proven relatively simple and very successful. Sitka spruce logs are more suitable for this purpose as they yield higher numbers of parasites. This research has proven that in the absence on an artificial host for *B. hylobii* that an alternative breeding system can be used. This system can be used to breed large numbers of *B. hylobii* which may be needed in any further research on the wasps. Using cut logs and with minor adaptations to the methods outlined in this research, a continuous breeding program (similar to Millar 2002) of both *H. abietis* and *B. hylobii* can be developed. Perhaps the best benefit of using this system is it greatly reduces the need to strip stumps in the field in search of either *H.*

abietis larvae or *B. hylobii* cocoons. With further efforts the system could be modified in regard to the size and types of logs used, timing of introduction of weevils and wasps, timing of stripping and optimal temperature for both weevils and wasps. This warrants further investigation.

Development from *B. hylobii* egg to cocoon stage in breeding chambers in the lab takes approx 14-16 days at room temperature with adults emerging shortly afterwards. *Bracon hylobii* cocoons, larvae and eggs were present in the same log at the same time after 17 days (chapter 8 and 9). This indicates a prolonged period of oviposition, which could produce some overlapping of generations. This may be a beneficial trait in the wasp as it would lead to a longer period of adult *B. hylobii* activity i.e. as older wasps die they are replaced by adults which developed later. This overlap of generations was also observed in *H. abietis* in the field. Adults, pupae, and larvae of *H. abietis* were observed in the same stump in field sites. In fact all stages of development of both *H. abietis* and *B. hylobii* were observed in the same stump approximately 1 year after felling. This may have implications for the successful control of *H. abietis* with *B. hylobii* as it suggests that it is poorly synchronised with its host a point also noted by Henry (1995) in his research.

Destructive sampling of logs in the field has shown higher percent parasitism rates by *B. hylobii* in Lodgepole pine stumps compared to Sitka spruce stumps. However in breeding chambers in the lab percent parasitism is consistently higher in Sitka spruce logs compared to Lodgepole pine logs. Reasons for this are most likely due to a combination of host abundance and availability and parasitoid dispersal. On field sites used in this program of research Lodgepole pine sites always had higher numbers of *H. abietis* larvae than Sitka spruce sites. This is not unexpected as pine is the natural host plant for *H. abietis*. If an abundance of hosts are present it is reasonable to presume that *B. hylobii* females will encounter hosts more frequently when searching. This may retain the females on site as they are obtaining a higher host reward for a given searching time. In Sitka spruce sites where host numbers are low there will be a greater searching time per host and also a higher likelihood of finding hosts that are already parasitised, this may entice the females to disperse to other sites. In the lab when equal or similar numbers of hosts were available in both

pine and spruce logs it is thought that the higher rates of parasitism found in the spruce logs was due to the thinner spruce bark compared to pine. The thinner bark is thought to be easier for the female *B. hylobii* to oviposit through and also the *H. abietis* larvae are closer to the surface in thinner bark. In nature, perhaps to avoid competition, predators and parasitoids of the same host often develop in different parts of the host habitat and in the case of parasitoids, that have to penetrate bark with their ovipositor, thinner bark is usually preferred (Wermelinger 2002). Research on two parasitoids *Syngaster lepidus* Brullé and *Callibracon limbatus* (Brullé) (Hymenoptera: Braconidae), which attack the eucalyptus longhorned borer, *Phoracantha semipunctata* F. (Coleoptera: Cerambycidae found that the species with the longer ovipositor was able to attack hosts concealed under thicker bark but that bark of a thickness greater than 17mm prevent either species from attacking the underlying host (Hanks *et al.* 2001). Female *B. hylobii* may also find it easier to locate host in thinner bark as short range cues such as host vibrations may be easier to detect where the host is concealed by a thin layer of bark. However in the field hosts are often parasitised deep within the thick bark of Lodgepole pine stumps. This indicates that host abundance and therefore wasp retention on site is more important than host accessibility. It may also indicate that in stumps of thick bark the female travels through holes, gaps or galleries in the bark to attack its hosts (pers. ob). Another point to consider in relation to the field rates of parasitism within pine and spruce is the initial attraction of *B. hylobii* to a site. As *B. hylobii* is a specialist parasitoid of *H. abietis* it most likely has an inherent attraction to the host's natural habitat, i.e. pine forests. This could mean that background populations of the wasp are higher in pine than spruce forests and therefore more capable of exploiting an increase in host numbers caused by clearfelling. However the reasonably low natural rate of parasitism by *B. hylobii* in the field on both pine and spruce clearfell sites suggests that background populations of the wasp in any case are not of a high enough level to fully exploit the available host resource. Further research into the background populations of *B. hylobii* in standing pine and spruce forests would be worth while

In lab experiments (chapter 8) *B. hylobii* females seem to be reluctant to parasitise hosts below the soil layer only doing so when large numbers of females are released

into a confined chamber. However these females are confined in the chamber and so the question remains, if they had the choice would they disperse when all easily attacked hosts are utilised rather than expend energy in attacking hosts, which are more difficult to locate? These observations indicate that given a choice in the field, when all 'easily' located hosts are used, dispersal is the favoured response. If this is the case it has obvious implications for the successful use of *B. hylobii* as a control agent against *H. abietis.* The nature of *H. abietis* development is that the larvae are often found on stumps in what would be perceived as difficult locations for *B .hylobii* to attack. However it is not the ability of the wasp to attack difficult to locate larvae, that is the problem, as larvae in these locations have been recorded as been parasitised (Henry 1995; Henry and Day 2001; pers. ob.). However it is the question as to whether the wasp will put the energy into searching for these larvae before dispersion that needs to be answered. If it is the case that females disperse after a certain amount of searching without finding a host, or at least an easy accessible host, then its potential as a control agent is questionable. The dispersal cues of *B. hylobii* from a site with available hosts needs to be investigated.

One theory to explain the dispersal of parasitoids from a site where hosts are available is that they leave in search of food especially if the host habitat is deprived of food (Takasu and Lewis 1995; Lewis *et al.* 1998). Whether *B. hylobii* females decide to search for food or hosts on emergence is of vital importance, however little is known about the immediate activity of these newly emergent females. In the context of a clearfell site, where there may not be an abundance of flowering plants (food), the choices made are of even greater consequence. On such sites, if females place the need for food over the need to parasitise, migration away from the site will result. This program of research indicates that unfed newly emerged females do in fact search for food before hosts (chapter 5). This being the case, possible solutions to promote the retention of wasps will be needed on sites where the available food source is low. This may be achieved through the establishment of flowering plants prior to any mass release or though feeding of wasps before their release. Both of these solutions have been attempted during this research, all be it on a small scale, but no real success was achieved. The addition of flowering plants has proven successful in other research programs (summarised in Wratten *et al.* 2002) and as

such deserves further research with regards to *B. hylobii*. Feeding wasps prior to release on a large scale is not feasible however the adaptation of a release vessel where newly emerged wasps would have a food source available may be worth investigating.

Although not statistically significant, fed LP/SS reared wasps seems to be attracted to *H. abietis* larvae feeding on LP and SS respectively. However there was also a large proportion in each case attracted to the empty chamber, indicating an innate need to disperse from the brood site before searching for hosts (chapter 5). To date little is known about the cues used by *B. hylobii* to find hosts. This research may indicate that the substrate from which the wasp emerged conditions its searching behaviour. Faccoli and Henry (2003) found that, in relation to searching for hosts, only the host feeding on bark produced searching behaviour by both naïve and mated female *B. hylobii*. They also suggest that as females gain experience from searching that their ability to find hosts improves. If both the searching experience and breeding substrate do in fact influence the searching behaviour of females, then modification to a future breeding program could be made. For example allowing females to 'learn' to associate hosts with the bark of the dominant tree species in the release site may be beneficial. In conjunction wasps reared in SS substrate may also be more successful on SS sites. A larger scale study to determine if this conditioning would increase wasp retention and parasitism levels on a target area needs further research.

Another point to consider in this research is that there is anecdotal evidence that the life cycle of *H. abietis* is shorter in Ireland compared to such places as northern Scotland (Munro 1929; pers. ob.) where winters are generally colder. Milder winters in Ireland could mean that *H. abietis* has a longer period within the season to develop before cold soil temperatures halt development. If this is the case, and particularly if background populations of *B. hylobii* are low, greater numbers of *H. abietis* may have developed past susceptibility to *B. hylobii* before the wasp has established itself in a new site. A more detailed study of the life cycle of *H. abietis* in Irish conditions and a further investigation into how well the two species are synchronised would yield useful information.

A range of flowering plants were tested for their attractiveness to *B. hylobii*. There appeared to be no significant preference for any of the tested flowering plants, which suggests more of a reliance on the most abundant food source on site at a given time of year rather than any one particular plant (chapter 4). Foraging on a variety of plants for food by *B. hylobii* would be a beneficial trait in regards to its longevity and thus its potential as a control agent. Clearfell sites, which are not *B. hylobii's* natural habitat, can often be devoid of a wide variety of flowering plants, particularly the year after clearfell, as it takes time for flowering plants to colonise the newly exposed sites. This could be an important factor as when/if *B. hylobii* migrate to a site in the year after clearfell there may be hosts available but an inadequate food source. This could result in a shorter lifespan in the adults that migrate to the site or it may result in dispersion away for the site for food (Takasu and Lewis 1995; Lewis *et al.* 1998; Wäckers 2002; Wratten *et al.* 2003; Lee *et al.* 2004). A decrease in adult longevity or an increase in dispersion will obviously have a negative effect on parasitism rates. Both the longevity experiment (chapter 6) and the experiment carried out to assess the effect of larval food source and non-host food on the searching behaviour of adult *B. hylobii* (chapter 5) support this view. Those experiments showed that the adult wasp's longevity can be extended when they have access to a regular food source and that newly emerged or un-fed wasps put the need to feed above the need to parasitise. In conclusion an early stage clearfell, lacking a food resource for adult *B. hylobii* thus decreasing longevity or promoting dispersal, may not be conducive to high rates of parasitism by *B. hylobii*.

Interference/disruption of parasitism by superparasitism or hyperparasitism may play a significant role in the lack of success of a parasitoid from a Family (Braconidae) that is usually an effective control. However molecular analysis of samples, from emergence traps that showed great variation in body size and number of antennal segments, showed that there was no difference in DNA. Evidence of superparasitism was, however, observed in the lab. If superparasitism is occurring in the field the energy consumed by the wasp by parasitising already parasitised hosts will decrease its ability to maximise its parasitism rate. This of course will have negative effects on the overall potential of the species as a control agent. The occurrence and frequency of superparasitism in the field should be investigated further.

Unfortunately predicting if and when *B. hylobii* will enter diapause is difficult. Henry (2000), found that only 5% of parasitoid cocoons collected from the field in mid July, when daily mean soil temperatures peaked at 15°C, had entered diapause. This was compared to 50% diapause rate in laboratory reared parasitoid cocoons at the same temperature. A mean temperature of 10°C in the laboratory caused 100% diapause. Henry concludes that maximum daily temperature is therefore likely to be responsible for diapause initiation.

When transferring parasitoid cocoons to the field, in an attempt to augment populations, it would be vital to know whether or not the parasitoid cocoons could enter diapause post transferral i.e. have the parasitoid cocoons gone past the point at which diapause could be initiated by cooler temperatures. This problem may have been encountered with the 'Lab infective units' in the field enhancement experiment (chapter 8). A sample of the 'Infective units' were stripped before they were introduced into the field. They all contained large numbers of cocoons and so the decision to move the remaining logs to the field was taken (August 05). The decision proved to be right at the time as wasps emerged from cocoons, that were held in the lab from the sampled logs, just days later. However when the logs were stripped the following February many of them contained a large number of full cocoons. This suggests that the wasps in the cocoons entered diapause upon transfer to field sites or they emerged and parasitised some of the remaining weevil larvae in the 'infective units' and it is these larvae that entered diapause and gave rise to the full cocoons in the following spring. *Ad hoc* samples of diapaused cocoons dissected in the lab contained *B. hylobii*, larvae, pupae and adults. However dissection destroyed the sample so it was not possible to know if those containing adults would actually continue development post diapause or were they pre-emergent adults killed by cold temperature. Knowing the point at which diapause in *B. hylobii* cocoons can no longer be initiated would aid in the corrected timing of release of pre-diapause augmentative populations. An alternative solution to this would be to breed the wasps in the autumn and allow them to enter diapause in the field, or the lab, if there is a cool room facility and then introduce them into a site that is approximately 1-year post felling the following spring so they emerge in the field that spring/summer.

Alternatively the wasps could be bred in late spring/early summer, when temperature should be high enough to prevent diapause, and introduced into the field once cocoons have formed.

Rates of parasitism in both artificial clearfell experiments (chapter 9) were low. Timing of the first experiment (logs transferred in April) may have been largely responsible for the low rates achieved. The points outlined previously in relation to diapause initiation may have been a factor. In the second experiment (adults released in July) most of the *B. hylobii* recorded on the parasitsted weevil larvae (approx 3 weeks after release) were still in the early stages of development. This would suggest that the wasps released may have either moved away from the site possibly in search of food returning at a later date to search for hosts or that searching and oviposition took place over a prolonged period. These experiments again reflect the problem off wasp retention on site.

The enhancement of percent parasitism achieved by *B. hylobii* in the field through the addition of logs containing *B. hylobii* cocoons proved unsuccessful (chapter 8). This could be due to a range of factors previously discussed such as diapause initiation, adult food resources and dispersal. In the case of transferring logs containing cocoons, if the logs are moved from the lab to site towards the end of the summer there is a possibility that many of them, when moving from the warm lab to the cooler outdoors, will enter diapause. The wasps will then emerge the following spring/summer, however if they were placed on an older site to start with then there is a possibility that the weevils will have developed past the stage which is susceptible to attack by the wasps by the time of emergence. A lack of a food resource on site, could lead to the wasps having to forage in sites away from where they emerged. As discussed, if the distance from emergence site and foraging site is too great or if there are hosts available in the foraging site the wasps may not return to the original site of emergence, whether there are hosts available, or not, in that site

Even though attempts to enhance the rate of parasitism in the field during this research were unsuccessful results from the lab were more positive. In breading chambers in the lab the rate of parasitism increased gradually with increasing

numbers of females from 8.35% to 83.19% for the SS logs and from 29.47% to 72.18 % for the LP logs. Some logs had 100% of the available larvae parasitised. These results also confirmed that, in the lab, SS proved better for breeding *B. hylobii*. These results prove that, in lab conditions, parasitism can be enhanced by the addition of greater numbers of the control agent. This, combined with the high levels of parasitism achieved, indicates that there is still potential in the field to enhance parasitism, should factors that negatively impact on it be identified and reduced and factors that benefit it be improved.

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13 Appendix

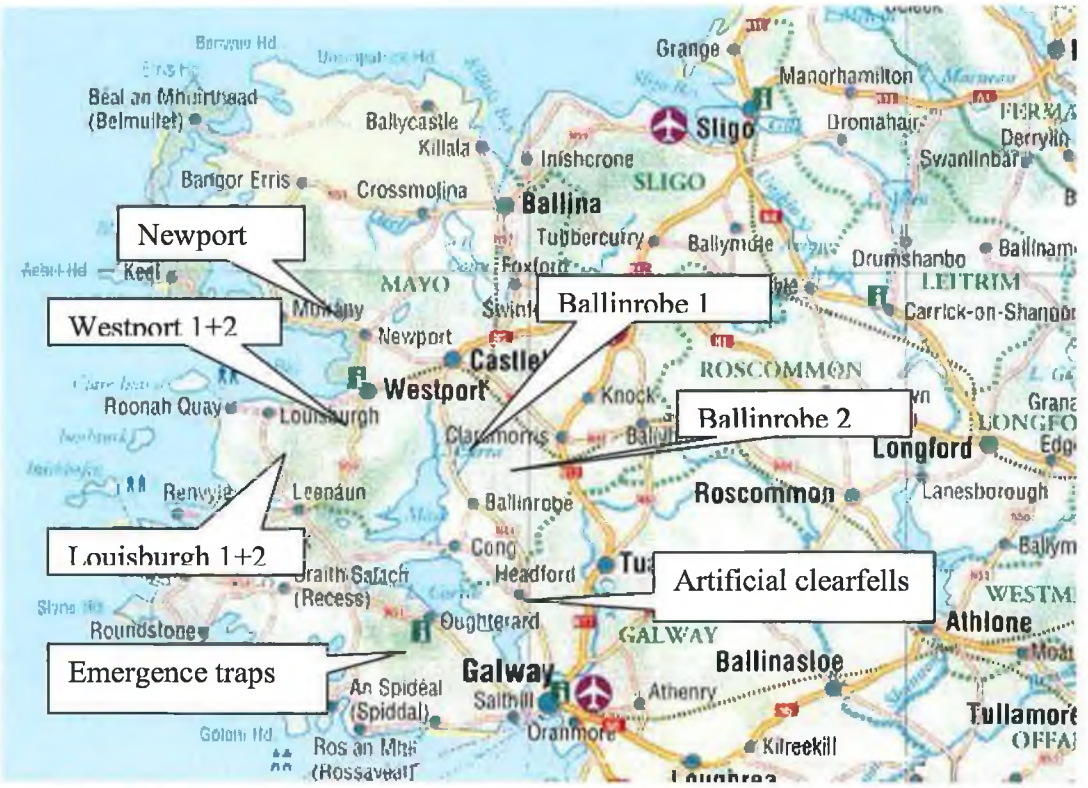

Figure 13.1. Map of field site locations