OLFACTORY AND VISUAL CUES IN HOST FINDING

BY BARK BEETLES

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ABSTRACT

This study examined the role of olfactory and visual cues in host finding of the pine bark beetles *Hylastes ater* and *Hylurgus ligniperda* and the burnt pine longhorn beetle *Arhopalus ferus*. The ultimate aim of this research was to provide new information on attractant and repellent (such as non-host leaf volatiles) stimuli to improve monitoring methods and reduce the attack by wood-boring and bark beetle species.

A field trapping trial of visual and olfactory cues near Nelson caught 7842 *H. ater*, 274,594 *H. ligniperda* and 16,301 *A. ferus* adults. There were significant effects of both visual (colour and sihoutte) and olfactory (host and non-host volatiles) cues for all three species. The highest catches were in black (host mimicking), panel flight intercept traps baited with attractant (α-pinene and ethanol) and the lowest in clear or white (non-host mimicking) control traps. The repellent, green leaf volatiles (GLV) ((E)-2-hexen-1-ol & (Z)-3-hexen-1-ol) when present on traps with attractant reduced catches significantly but modestly in *H. ater* and *H. ligniperda*, but had no significant effect on *A. ferus*.

A field trial near Christchurch found that GLV applied as a topical repellent halved the number of beetles attacking *Pinus radiata* logs. This reduction was significant in *H. ligniperda*, but not quite ($P = 0.07$) in *H. ater*. Placing logs among broadleaved plants (natural sources of non-host volatiles) significantly reduced attack of *H. ligniperda* by about 75% compared to logs in the open, but had no effect on *H. ater*.

Attack by *H. ater* was found on 4% of 500 *P. radiata* seedlings in a field trial near Dunedin. Treatment of seedlings with GLV significantly affected the severity and proportion of seedlings attacked by *H. ater*, compared with insecticide-treated and control seedlings, but the treatment effect was apparently driven by an unexpected direct damaging effect of GLV on the health of seedlings.

It is recommended that future research explores the use of non-host volatiles from natural sources that influence host finding in wood–boring and bark beetle species for the protection of plantation forests in New Zealand.

1   GENERAL INTRODUCTION

Coniferous tree species make up the majority of New Zealand plantation forests which cover approximately 1.8 million hectares. The primary plantation species, *Pinus radiata* D. Don (Coniferales: Pinaceae) accounts for approximately 1.6 million hectares and 19% of New Zealand's total forest area (Annon, 2010). To meet New Zealand's timber requirements each year, a large expanse of plantation forests are harvested by clearfelling. For example, in 2009 an estimated 41 thousand hectares of plantation forest was harvested (Annon, 2010), which leaves substantial amounts of woody debris behind.

Beetles (Coleoptera) in the family Scolytidae (Atkins, 1966) (now considered a subfamily, Scolytinae) and Cerambycidae (Allison, et al., 2004) occupy tempory habitats within debris of forest environments. Ecologically these species form diverse groups that play many important roles in natural ecosystems (Milligan, 1975; S. L. Wood, 1982; Raffa & Berryman, 1983; Raffa, et al., 1993; Safranyik, 1995; Knizek & Beaver, 2004). A large proportion of the Scolytinae and Cerambycidae are subcortical-feeding and wood-boring insects which include some 6000 species (Kirkendall, 1983; Raffa, et al., 1993; Byers, 2004; Knizek & Beaver, 2004), and 35,000 species (Allison, et al., 2004), respectively, world wide. Within Scolytinae there are an estimated 500 species that are asscociated with coniferous tree species (Seybold, et al., 2006). Wood and bark boring insects form one of the most difficult groups of forest and timber insect pests, with 11 exotic species established in New Zealand (Brockerhoff, et al., 2003). New Zealand's native biodiversity is continuously under threat from new introductions of potential pests and invasive species (Liebhold, et al., 1995; Brockerhoff, et al., 2006b; Brockerhoff, et

al., 2010a; Brockerhoff, et al., 2010b). An organism is considered a pest or invasive if, when expanding its natural range, it impacts detrimentally on other organisms, threatens economic resources or human health (Liebhold, et al., 1995; Foster and & Harris, 1997; Brockerhoff, et al., 2010a).

The majority of bark beetle species colonise the subcortical region under the bark of dead, dying or stressed trees, although some will attack and kill living healthy trees in optimum conditions becoming devestating and invasive pests. This subcortical lifestyle represents considerable difficulties to managment, and bark beetles are considered one of the economically most important insect problems to forestry world wide (Rudinsky, 1962; Milligan, 1975; S. L. Wood, 1982; Ciesla, 1988; Raffa, et al., 1993; Kurz, et al., 2008).

Plant-feeding insects vary in terms of their host specificity, albeit on a continuous (not binary) scale. Polyphagous insects can exploit numerous plant species (though often more or less closely related ones), whereas oligophagous and monophagous insects are much more specialised, restricted to one or a few closely related species (Bertheau, et al., 2010). Bark beetles have evolved forms to exploit every type of plant tissue. Most species are either phloeophagous, where they feed on and utilise the inner bark, phloem and cambium region of woody plants, or xylo-mycetophagous, with all life stages feeding on mutualistic fungi that grow on sap or heartwood of the host tree (Milligan, 1975; Kirkendall, 1983; Raffa, et al., 1993; Sauvard, 2004). The life cycle of many scolytid species is completed within the bark of the host, with the exception of a period of dispersal when adults take flight after they emerge from brood material in order to colonise new areas (Rudinsky, 1962; Atkins, 1966; D. L. Wood, 1982; S. L. Wood, 1982; Raffa & Berryman, 1983; Faulds, 1989; May, 1993). Bark and wood boring species are

characterised according to the type of trees they colonise which are either alive or dead/dying as aggressive or non-aggressive species. Aggressive species, also known as near obligate parasites (Raffa, et al., 1993) or primary species (Rudinsky, 1962), are those that are regular tree killers, often colonising healthy, living trees. Non-aggressive or secondary species, (Rudinsky, 1962) which cover the majority of Scolytinae, can be divided into two catagories. Firstly, facultative parasites (Raffa, et al., 1993) are species that normally colonise fallen, windthrown trees or cut logs, but in times where conditions facilitate increased population numbers they can colonise living trees which are weakened by drought, fire, age, fungus, or competition (Rudinsky, 1962). Secondly, the herbivore/saprophyte (Raffa, et al., 1993) or saprophagous species (Rudinsky, 1962) are those which colonise material that is generally aged and show some fermentation. Such material can include dead trees killed by any of a number of factors, old logs or windthrown trees, all of which can be a rare and unpredictable resourse in a natural forest environments (Raffa, et al., 1993). In plantation or monoculture situations when resources are readily available, secondary bark beetle adults will colonise harvesting by-products and waste wood as well as felled logs on skid sites, causing direct damage by boring into the wood, creating tunnels and galleries and allowing fungi under the bark which can degrade and stain wood and create export quarantine risks (Milligan, 1978; May, 1993; Knizek & Beaver, 2004). These life-history traits increase the likelyhood that wood-boring and bark beetle species will remain undetected on export logs. Therefore measures are taken to prevent the transportation of stow-away beetles with exports of forest products, to limit potential new introductions into other countries (Brockerhoff, et al., 2006a). The impact of an introduced species creates a high economical and environmental

cost, disrupting foreign ecosystems and directly impacting forest industry through degrading wood or killing host trees (Brockerhoff, et al., 2006a).

This thesis focuses on three important wood-boring and bark beetle species, the black pine bark beetle *Hylastes ater* (Payk.) (Coleoptera: Scolytinae), the golden-haired bark beetle *Hylurgus ligniperda* (Fabr.) (Coleoptera: Scolytinae), and the burnt pine longhorn beetle *Arhopalus ferus* (Muls.) (Coleoptera: Cerambycidae). All three are European pests of coniferous tree species which have arrived and become established in New Zealand. These three species account for the majority of individual exotic bark beetles and wood borers caught in New Zealand, and they are among the most economically important invasive forest and timber insect pests (Faulds, 1989; Brockerhoff, et al., 2006b).

Hylastes ater, *H. ligniperda* and *A. ferus* are generally secondary, non-aggressive species that breed mainly in cut tree stumps, roots and wood of pine trees that remain after harvesting of New Zealand's plantation trees. As a result, these insects are often extremely abundant in pine plantation regions, particularly in the years following tree harvesting. These insects can cause direct damage through brood galleries and feeding tunnels formed under the bark of harvested or wind thrown logs, introducing sapstain and decay fungi which can reduce the quality and value of logs if not promptly processed.

1.1   The black pine bark beetle (*Hylastes ater* (Paykull))

The bark beetle genus *Hylastes* comprises around 30 species (S. L. Wood, 1982), a few of which are considered to cause significant problems in forestry (Clark, 1932; Tribe, 1991; Lindelöw, 1992; Liebhold, et al., 1995). The black pine bark beetle, *Hylastes ater*, is shining black in colour, 4-5mm long and 1.4mm wide (Milligan, 1978). This native to Europe, which occurs across Europe from Spain to Russia (Clark, 1932; Milligan, 1978), has been introduced into Australia, New Zealand, and Chile (Milligan, 1978; Ciesla, 1988). The predominant hosts of *H. ater* are *Pinus* species (Pineaceae), in New Zealand mainly *P. radiata,* although they are also known to attack *Picea* species (spruce), *Abies* species (fir), *Pseudotsuga menziesii* (Douglas fir), and *Larix decidua* (larch) (Milligan, 1978).

In New Zealand, forest entomologist Arthur Clark of the State Forest Service first reported *H. ater* (Clark, 1932), receiving a specimen along with a damaged *P. radiata* seedling in August 1929 which originated from Foxton, North Island. *Hylastes ater* is now established throughout New Zealand within regions of exotic plantation forests. This species has been noted in New Zealand as the most troublesome insect in *P. radiata* regeneration due to the destruction it can cause when feeding on the root collar of seedling plants (Zondag, 1965). *Hylastes ater* often co-occurs with the larger and apparently, more aggressive *Hylurgus ligniperda,* in New Zealand and Chile (Ciesla, 1988). The extent to which competition between them affects the population dynamics of these two species is unknown. In Europe it is suggested that species of *Hylastes* are

restricted by species of *Hylobius*, weevils (Curculionidae) which generally out-compete *Hylastes* for breeding material (Lindelöw, 1992).

In Australia and New Zealand, adults and larvae are present year round (Clark, 1932; Crowhurst, 1969), and the life cycle takes 60 – 300 days from egg to adult, depending on time of year and conditions (Milligan, 1978). In Britain there are two generations a year, but in New Zealand there can be up to three generations a year that may overlap if conditions are favourable (Clark, 1932). Adults of *Hylastes* species create feeding tunnels and brood galleries in the phloem and cambium region under the bark of the roots, stumps and felled or damaged trees or logging waste, preferring the underside that is in contact with the ground. Newly emerged adults will also attack the lower stems, roots and root collars of seedling trees in maturation feeding, having detrimental effects for forest establishment in New Zealand and elsewhere. In England, damage was recorded on trees up to 6 years old, and 10 years in Germany (Clark, 1932; Boomsma & Adams, 1943; Zondag, 1965; Milligan, 1978; Lindelöw, 1992; Reay & Walsh, 2002b). It is rare that *H. ater* kills healthy seedlings that it attacks; the most drastic damage is done in second rotation forests (Clark, 1932; Zondag, 1965; Milligan, 1978; Reay & Walsh, 2002b). The cost of avoiding the damage caused by maturation feeding has been identified by the New Zealand forestry industry as the most signficant economic impact of any forest insect pest. *Hylastes ater* is considered an important threat to the biosecurity of all countries with conifer forests (Brockerhoff, et al., 2006b) as a secondary pest attacking and degrading timber, damaging seedling trees and as a vector for plant-pathogenic and lumber sapstaining fungi (Reay & Walsh, 2002b; Reay, et al., 2002; Reay, et al., 2005; Mausel, et al., 2007).

1.2 The golden-haired pine bark beetle (*Hylurgus ligniperda* (Fabricius))

The golden- or red-haired pine bark beetle, *Hylurgus ligniperda* is 7 - 8 mm long and 2 mm wide, dark brown or black in colour with numerous yellow setae on its elytra (Bain, 1977). Under normal conditions, the life cycle of *H. ligniperda* takes 10 to 11 weeks from egg to emerging adult (Bain, 1977), which disperse in two peaks of adult flight activity during the spring and autumn, corresponding to the two generations per year that are typical in New Zealand. Adults invade and breed in fresh host material including cut stumps, logs and slash of trees following harvesting.

Hylurgus ligniperda is native to central Europe**,** Asia Minor, and Mediterranean regions, and introduced into Japan, Sri Lanka, South Africa, Australia, New Zealand, Brazil, Chile and North America (Bain, 1977; Lee, et al., 2007). Its predominant hosts are *Pinus* species (Bain, 1977; Lee, et al., 2007). The first record of *H. ligniperda* in New Zealand was in 1974 near Whitford, south of Auckland (Bain, 1977), and it is now found in forested regions throughout the North and much of the South Island.

Hylurgus ligniperda is found in similar situations as *H. ater* causing similar problems, such as adults creating brood galleries and larvae living in the inner bark of logs, felled timber and in tree material, that are predominantly in contact with the ground (Faulds, 1989; Tribe, 1991). However, adults have not been known to attack seedlings in New Zealand (unlike *H. ater*). The main type of damage in forests is from wood-staining and decay fungal associations that enter with the adults into the brood galleries (Tribe, 1991), and as a quarantine pest that may necessitate treatment of export logs and timber.

1.3   The burnt pine long horn beetle (*Arhopalus ferus* (Mulsant))

Species in the genus *Arhopalus* (Coleoptera: Cerambycidae) are widespread across much of the northern hemisphere and associated with coniferous tree species (Wang & Leschen, 2003). Cerambycid beetles (or longhorn, long-horned or longicorn beetles) are becoming increasingly important pests worldwide, initiating wood degradation in forests (Allison, et al., 2004). The burnt pine longhorn beetle, *Arhopalus ferus* is distributed throughout much of Europe, northern Asia (except Japan), and northernmost Africa (Brockerhoff & Hosking, 2001). An introduced species to New Zealand, it was first discovered in 1963 in fire-killed *P. radiata* at Mamaranui, North of Auckland, but the accidental introduction was suggested to have occurred already in the 1950s (Hosking & Bain, 1977). *Arhopalus ferus* has spread throughout the North Island and much of the South Island of New Zealand (Brockerhoff & Hosking, 2001).

Adult beetles are brown to black in colour and from 12 to 30 mm in length, the life cycle in New Zealand taking 1 to 2 years. The larval stages live in the inner bark and may enter the sap wood. *Arhopalus ferus* larvae are strongly influenced by intra-specific competition and temperature during this stage (Bradbury, 1998; Brockerhoff & Hosking, 2001). Adults emerge around 50 days after larvae pupate, living for several weeks between November to March, and are active in the dusk through the early evening (Brockerhoff & Hosking, 2001).

The predominant hosts in New Zealand are at least eight *Pinus* species, and, less commonly, also *Pseudotsuga menziesii* (Douglas fir) and *Larix decidua* (European larch).

In Europe, *Picea abies* (Norway spruce) is the predominant host species (Brockerhoff & Hosking, 2001).

Arhopalus ferus does not usually cause high levels of damage in forests, but it can show rapid attack after fire events which limits forestry salvage times (Hosking & Bain, 1977; Bradbury, 1998; Wang & Leschen, 2003). Adults have been recorded attacking logs, stumps, and standing dead or damaged trees (Brockerhoff & Hosking, 2001), and are known to vector sapstain and decay fungi such as *Ophiostoma* species (Bradbury, 1998; Suckling, et al., 1999). *Arhopalus ferus* has become a quarantine issue due to high numbers of adults attracted to saw mills and port areas where timber is stored, as beetles shelter among sawn timber destined for export (FRI, 1973; Hosking & Bain, 1977; Brockerhoff & Hosking, 2001).

Due to the occasional presence of *A. ferus* and other beetle species on export timber, chemical controls are required using fumigants such as methyl bromide, preservatives and insecticides (FRI, 1973) to treat wood for export. These chemical are now known to impact on human health and alternatives are being investigated (Brockerhoff & Hosking, 2001).

1.4   Host finding in bark beetles

Regardless of their life-history traits, all bark beetles require non-resistant, recently dead host material for brood production (Raffa, et al., 1993). The ability of adults to find suitable host material becomes a limiting factor for bark beetles (Lindelöw, et al., 1992; Knizek & Beaver, 2004). Selection for primary attraction and efficient searching mechanisms to find suitable host material are therefore critical for individual fitness (Alcock, 1982; De Jong & Sabelis, 1988; Tunset, et al., 1993). In 'monoculture' forests, host material can be abundant, allowing bark beetles to exploit the resources for a rapid increase in population numbers. However, even though host material can be plentiful, most breeding material is often only suitable for beetle feeding for one season after harvest (Lindelöw, 1992). Over time the phloem under the bark dies, and therefore successive generations must find new host material, often by dispersing to new areas. D.L. Wood (1982) suggested four classifications for the phases of host colonisation by bark beetles: dispersal, selection, concentration and establishment. The phase of dispersal is important in understanding host-finding behaviour, beginning with emergence of young or over-wintering adults which move away form the brood host material, ending with a response to host-specific cues from volatiles or pheromones (D. L. Wood, 1982; Raffa, et al., 1993). Many bark beetles of coniferous tree species are primarily attracted to host material by olfaction during dispersal, utilising host-specific volatiles for recognition and orientation (D. L. Wood, 1982; Schroeder & Lindelöw, 1989; Lindelöw, et al., 1993; Reay & Walsh, 2002c; Brockerhoff, et al., 2006b; Miller & Rabaglia, 2009). Insects have been shown to respond at some distance to plant olfactory stimuli, and then at close range, to respond to visual stimuli (Prokopy & Owens, 1983). In herbivorous insects, the hue

(colour) and intensity (brightness) of the plant appear to be the principle stimuli that aid in orientation for detecting plants at close range, at least partly on the basis of the host's attractive properties such as dimension or growth pattern characteristics. For example, bark beetles respond positively to tall, narrow, objects like vertically growing stems (Prokopy & Owens, 1983). Understanding bark beetle host finding behaviour offers opportunities for management with the combination of known attractive olfactory and visual cues used to increase catch in monitoring traps, allowing for better estimates of populations and monitoring of trends which are critical for effective management and assessment.

Inter-planting of known host species with other vegetation or planting mixed stands of trees in forestry has shown to result in fewer insect damage-causing pest outbreaks than in monoculture forests (Prokopy & Owens, 1983; Jactel, et al., 2005; Jactel & Brockerhoff, 2007). In mixed forests, the available host material can be unevenly distributed in space and time (Atkins, 1966). Disruption in host finding in mixed forests has been suggested to be due to the presence of non-host tree species creating an olfactory barrier (Q. H. Zhang & Schlyter, 2004) and presumably also a physical or visual barrier in limiting host-specific cues for insects. Research in Europe and North America demonstrates the effectiveness of non-host cues in disrupting host finding behaviour (Schroeder, 1992; Wilson, et al., 1996; Deglow & Borden, 1998; Q. H. Zhang, et al., 1999b; Byers, et al., 2000; Strom, et al., 2001; Huber & Borden, 2003; Byers, et al., 2004; Q. H. Zhang & Schlyter, 2004; Campbell & Borden, 2006b). The ability to exploit the mechanisms of host finding of bark beetles will ultimately help develop management strategies for a preventative approach (Raffa, et al., 1993).

1.5   Project Objectives

The project aims to discover the relative importance of the mechanisms and cues that are involved in host finding of *Hylastes ater*, *Hylurgus ligniperda* and *Arhopalus ferus* and how these beetles can be disrupted by presenting inappropriate (non-host) cues. The use of visual and olfactory host and non-host cues could provide 'greener' tools for managing these pests, by reducing or preventing attack of seedlings and timber.

The main objectives of this project are:

- to determine the roles of olfactory cues and visual cues in host finding of *Hylastes ater*, *Hylurgus ligniperda* and *Arhopalus ferus* in the field;

- to determine the influence of non-host volatiles that may act as repellents to *Hylastes ater*, *Hylurgus ligniperda* and *Arhopalus ferus* in the field;

- to explore whether mixed plantings can potentially provide sufficient non-host volatiles to have a repellent effect; and

- to contribute to the development of management techniques for these beetles that rely on the use of non-host volatiles (from dispensers or directly applied to plants, or emanating from non-host plants present among host plant material) to reduce attacks of pine seedlings, logs, and export timber.

The role of olfactory and visual cues in host finding in the bark and wood boring beetles, *Hylastes ater*, *Hylurgus ligniperda* and *Arhopalus ferus* will be assessed using several experimental approaches. Traps commonly used to monitor these insects - intended to

mimic hosts with visual and olfactory cues - can be modified to separate the different cues beetles use to find their host, thereby revealing the relative importance to that species. In addition, the role of inappropriate stimuli such as non-host volatiles and 'wrong' or missing visual cues can be tested to determine how strong the repellent effect may be. The results from these preliminary tests with non-host volatiles will then be verified with further field tests using log billets and seedlings with and without application of candidate factors thought to be repellent.

An additional aspect of this research is concerned with the potential of mixed plantings to provide non-host volatiles that may reduce the host-finding ability of bark beetles and thus the damage they cause to coniferous host material. This was done with a paired study that consists of log billets in an open environment or mixed with other plants that are known to emit non-host volatiles. Study sites suitable for such experiments exist, for example in areas where broadleaved 'weeds' are a substantial component of the vegetation amongst pine seedlings. A recent review documented the occurrence and scale of this 'pest control effect' of mixed plantings, compared with single-species plantings (Jactel & Brockerhoff, 2007).

2   (THE) ROLE OF OLFACTORY AND VISUAL CUES IN HOST FINDING BY *HYLASTES ATER, HYLURGUS LIGNIPERDA* AND *ARHOPALUS FERUS*

2.1   Introduction

Host specific wood boring and bark beetles species are highly adapted to find and infest potential hosts that can be widely distributed in natural forest environments, and respond strongly to increased host material produced by natural events, for example, storms and fire, utilising host specific visual and olfactory cues (Rudinsky, 1962; D. L. Wood, 1982; Raffa & Berryman, 1983; Raffa, et al., 1993; Campbell & Borden, 2006a). In the process of host selection bark beetles have the potential to disperse through flight some distance in order to find suitable host material (Atkins, 1966), for example, *A. ferus* is known to travel more than 3 km (Brockerhoff & Hosking, 2001), and *Ips typographus* up to 19 km (De Jong & Sabelis, 1988). Flying or dispersing individuals should progressively narrow their search for host material in response to environmental stimuli, from visual and olfactory host specific cues utilised in host finding behaviour to allow for the most effective searching (Strom, et al., 1999; Huber, et al., 2000).

It has been understood that foraging insects utilise olfactory cues from their host in a single sensory mode of host selection (D. L. Wood, 1982; Byers, 2004; Seybold, et al., 2006; Campbell & Borden, 2009). Generally, primary or aggressive species use host

volatiles in conjunction with aggregation pheromone typically produced by con-specifics colonising a host to organise mass attacks to overwhelm the defences of a chosen living host tree (Rudinsky, 1962; D. L. Wood, 1982; Raffa & Berryman, 1983; Raffa, et al., 1993). By contrast, secondary or non-aggressive species typically do not to use aggregation pheromones but focus solely on host specific cues to orientate to new host material (Rudinsky, 1962; D. L. Wood, 1982; Raffa & Berryman, 1983). In the complex visual and olfactory landscape within a forest, individual fitness may be increased during host selection through the incorporation of more than one sensory mode to discriminate hosts from non-hosts (Strom, et al., 1999; Campbell & Borden, 2009). It has been demonstrated that host selection is expected to favour the process that is most accurate and least costly, considering the visual capability, and host properties to allow host discrimination (Alcock, 1982; Prokopy & Owens, 1983; Jong & Sabelis, 1988; Tunset, et al., 1993; Campbell & Borden, 2006a).

Many conifer-colonising bark beetle species are noted to not only use characteristic volatiles to identify or discriminate host and non-hosts species (Campbell & Borden, 2009), but orientate and land on dark vertical objects, a specific characteristic of host trees (Strom, et al., 1999). Therefore aspects of visual host finding should not be readily separated from orientation to olfactory stimuli (Prokopy & Owens, 1983).

The black pine bark beetle (*Hylastes ater*), the golden haired bark beetle (*Hylurgus ligniperda*), and the burnt pine long-horn beetle (*Arhopalus ferus*) are essentially secondary non-aggressive bark beetles (in the case of *H. ater* and *H. ligniperda*) or wood borers (*A. ferus*) that are more or less specific to species of pine (*Pinus* spp.) and widely distributed throughout New Zealand. Previous research on *H. ater*, *H. ligniperda* and *A.*

ferus has proven that pine-specific host volatiles, presumably in conjunction with visual stimuli, play an important role in orientation and selection of host material (Reay, 2001; Suckling, et al., 2001; Reay & Walsh, 2002c; Brockerhoff, et al., 2006b).

There is undoubtedly a role of attractive host specific stimuli in host selection of bark beetles. Therefore, inappropriate olfactory stimuli such as non-host volatiles and 'wrong' or missing visual stimuli must influence host finding behaviour. Because the behaviour of insects is linked to their enviornment through mulitple sensory modes, there are mulitple opportunities to disrupt host finding through interference with sensory cues (Strom, et al., 1999). This has the potential to influence the pest management strategies for bark beetle species.

Research on wood boring and bark beetle species in New Zealand, Europe and North America demonstrates that non-host olfactory and visual stimuli can be effective in disrupting host-selection (Schroeder, 1992; Wilson, et al., 1996; Borden, et al., 1997; Byers, et al., 1998; Delglow & Borden, 1998; Strom, et al., 1999; Q. H. Zhang, et al., 1999a; Q. H. Zhang, et al., 1999b; Byers, et al., 2000; Huber, et al., 2000; Q. H. Zhang, et al., 2000; Borden, et al., 2001; Huber & Borden, 2001b, 2001a; Strom & Goyer, 2001; Strom, et al., 2001; Suckling, et al., 2001; Huber & Borden, 2003; Byers, 2004; Byers, et al., 2004; Goyer, et al., 2004; Q. H. Zhang & Schlyter, 2004; Campbell & Borden, 2006a, 2006b, 2009).

Experiments in olfactory discrimination or disruption of bark beetles utilise volatile chemicals emitted from non-host angiosperm species that are found in natural forest environments. For example, volatiles from species of *Populus* (aspen) and *Betula* (birch) have been analysed and tested for physiological and behavioural responses (Q. H. Zhang,

et al., 1999a; Q. H. Zhang, et al., 1999b). The visual characteristics of angiosperm species have also been measured through bark reflectance to show that there is spectral contrast between the bark of some angiosperms and the bark of coniferous tree species (Campbell & Borden, 2005, 2006a), when the visible spectrum of insects is considered, between ultra violet (350 nm) and red (650 nm) (Prokopy & Owens, 1983).

A better understanding of the host selection behaviours of *H. ater, H. ligniperda* and *A. ferus* would enhance our ability to develop more effective of management strategies based on manipulation of host attraction and disruption.

Monitoring the flight activity during the research project was undertaken to determine the relative abundance of these species within the Nelson region, where all three species of interest were present, and Dunedin where *H. ater* and *H. ligniperda* were present. The monitoring also aimed to identify the periods during which the three beetles are most actively dispersing and searching for new host material.

The aim of this research project was to investigate the role of olfactory and visual stimuli, and the interactions between these, in the host finding of *H. ater, H. ligniperda* and *A. ferus* through the manipulation of host and non-host specific cues during the period of peak activity. The objectives of this research project were therefore, to investigate for *H. ater, H. ligniperda* and *A. ferus*:

- the seasonal patterns of flight activity of the these beetles;

- identify times of enhanced flight activity during the day and year;

- to determine the roles of olfactory cues and visual cues in host finding of *Hylastes ater*, *Hylurgus ligniperda* and *Arhopalus ferus*;

- to assess the potential of non-host volatiles to act as repellents to host finding;

- to contribute to the development of a management technique for these beetles that relies on the use of non-host volatiles to reduce attacks of pine seedlings, logs, and export timber.

2.2   Methods

2.2.1   Seasonal and Daily Flight Activity

Seasonal Flight Activity

To monitor the seasonal flight activity, five sites were selected in third rotation *Pinus radiata* forest (Golden downs and Kainui Forests) in the Nelson region at the beginning of November 2008, the expected start of the target species' spring flight. Five additional sites were established across the region at the end of November 2008 in *P. radiata* forest (Moutere and Lee Valley Forests). The sites covered a wide geographical and environmental range across the region (Figure 2.1). All sites had been harvested within the previous twelve months according to information provided by Nelson Forests Ltd and Hancock Forest management.

Seasonal flight activity of *H. ater, H. ligniperda* and *A. ferus* was monitored at all ten sites which also accommodated the experimental trials testing visual and olfactory cues in host-finding of wood-boring and bark beetles. Traps selected for monitoring of flight activity represent two of twenty traps established at each site to test different host-finding cues. Monitoring trap types were a black panel trap and Lindgren type funnel trap, which were used to draw comparisons between catch rate of two different types of traps which were in place for two field seasons, discussed in the next section (2.2.2).
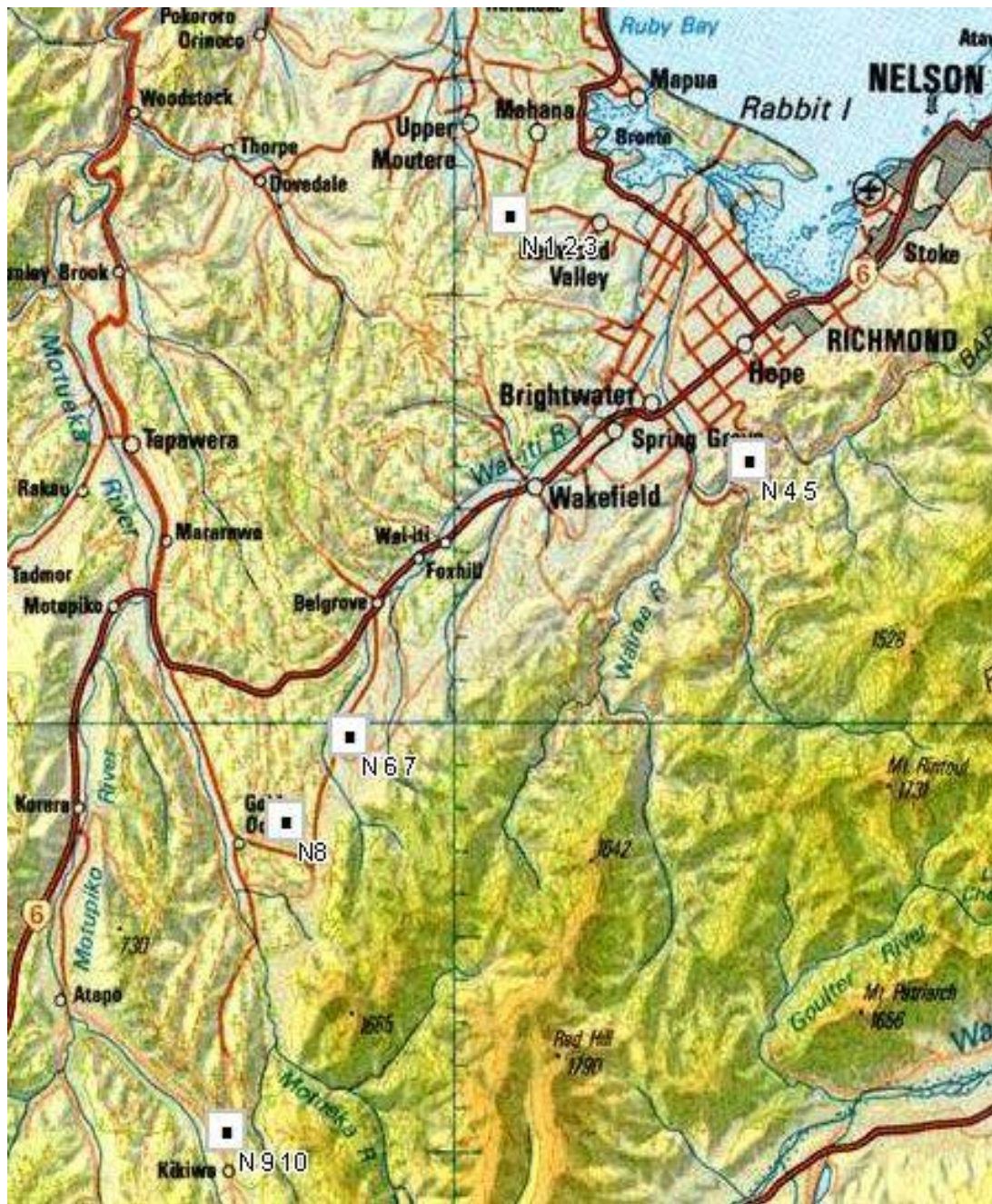
Figure 2.1 Site locations (grey and white squares mark sites, N – designates Nelson, followed by the site number at specific location) throughout *P. radiata* forests in the Nelson region, New Zealand. Map extracted from NZMS 1:500,000 series. For scale, the distance from N1 to N10 is about 40 km.

Flight activity of the three target species was monitored using Lindgren 8-funnel flight intercept traps (Phero Tech Inc., Delta, British Columbia, Canada) and custom-made black panel flight intercept traps, suspended from 1.6m steel posts installed on site. The Lindgren traps are commonly used commercially available monitoring traps that are black in colour and are thought to mimic the silhouette of host tree boles to foraging insects (Prokopy & Owens, 1983; Strom, et al., 1999) (Figure 2.2A). The new panel flight intercept traps were developed to be an economically more viable and experimentally more flexible design, available in a variety of different colours for further testing towards host finding cues (Figure 2.2B). The motivation for this is due to Lindgren traps only being available in black, therefore alternative traps were needed to be able to compare catch rate with trap colour. Monitoring with both traps allowed for a comparison of catch with the already proven Lindgren funnel trap to the new black panel trap.

Monitoring traps were baited with known attractive host volatiles (Reay & Walsh, 2002c; Brockerhoff, et al., 2006b) - 150 ml of alpha-pinene and 150 ml ethanol attractant in chemical dispensers attached to the side of each trap. Alpha-pinene (2,6,6-trimethylbicylo[3.1.1]hept-2-ene) (Hexion, Mt. Maunganui, New Zealand), at a minimum concentration of 95% and ethanol (ethyl alcohol), at a minimum concentration of 98% were used in two separate chemical dispensers made from sealed polyethylene tubes. Chemical dispensers for attractants and repellents (discussed below) were made at Scion (Christchurch, New Zealand) from, 400 x 50 mm (attractant) and 200 x 50 mm (repellent), 150 μm polyethylene lay-flat tubing (Accord Plastics, Masterton, New Zealand) fitted with felt strips (Fabric Vision Ltd, Christchurch). Felt was used to assist in even release of volatiles across the length of the tubing, as volatiles are absorbed along the felt within the polyethylene tube.

Traps were monitored on a fortnightly to monthly basis from first establishment on 7 November 2008 until 1 February 2010. Each monitoring time consisted of the removal of all insects caught per trap and storing them in -20 degree Celsius freezer until they were sorted and counted in the laboratory, where only the target species were recorded and by-catch discarded.

At the end of April 2009 site N4 in the Lee Valley forest, Nelson had to be removed due to logging in a nearby stand. This left 9 sites with a combined 18 traps available for continued monitoring during the year. In the winter months trap monitoring was reduced to once per month, then increased back to fortnightly in the spring.

Daily Flight Activity

To assess the hours of flight activity within the day of *H. ater, H. ligniperda* and *A. ferus*, traps were cleared every three hours over two 72 hour periods. Two established sites were selected for monitoring in Kainui Forest, Nelson. The aim of the daily trapping was to assess beetle activity and obtain a good indication of daily flight patterns during the height of the summer flight in 2009 and 2010. In the first season, monitoring of the 40 established traps started on 2 February 2009 at 1:30 pm and ran until 5 February 2009 at 10:30 am. Due to variable weather conditions over the trapping period data obtained varied substantially, so a second daily data set was collected in 2010 at the same two sites in Kainui Forest. Trap numbers at these sites had been reduced during the previous year to four traps for monitoring purposes. Trap monitoring started 18 January 2010 at 10:30 am and ran until 21 January 2010 at 1:30 pm. During both collection periods temperature was recorded at both sites at each monitoring time.

Data Analysis

All seasonal trap catch data were standardised by converting total trap catch to trap catch per trapping day.

Data for daily flight patterns were investigated using three QuasiPoisson generalized linear models for effects of year and time of day on the number of *H. ater, H. ligniperda* and *A. ferus* respectively. All data analysis was performed using the statistical package R, version 2.10.0 for Windows (R Development Core Team. 2009. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria).

Figure 2.2 A. Lindgren type Funnel Trap (Phero Tech Inc., Delta, British Columbia, Canada); **B**. Black Panel flight intercept trap made to the same dimensions of the Lindgren funnel trap. Height: 850mm x Width: 250mm (not including 125ml catch jar). Note; chemical dispensers for attractant (alpha-pinene and ethanol) on the side of the traps (see text).

2.2.2   Olfactory and Visual Cues in Host-finding

Nelson

The same 10 sites were used as previously described in section 2.1.1. Twenty traps used to test the effect on visual and olfactory cues in host finding of *H. ater, H. ligniperda* and *A. ferus* were established per site from 7 to 9 November 2008. The trial continued until 1 May 2009 when 18 of the 20 traps per site were removed, leaving two in place for continued flight monitoring during the remainder of the year. Trapping from November until the end of April was expected to cover most of the spring to autumn flight activity.

I tested whether *H. ater, H. ligniperda* and *A. ferus* discriminated among twenty trap types with host and non-host characteristics (Table 2.1.). Traps were placed at a minimum of 20 m apart suspended from wire attached to 1.6 m steel posts (Figure 2.2). Trap positions were in lines to suit the specific terrain. Traps were established so that no two traps of similar colour or treatment were positioned next to each other. To limit position effects, from environmental factors such as the variable distribution of known host material across the site, traps were moved one position clockwise along the line at each trap monitoring occasion. All traps were monitored on a fortnightly basis from trial establishment until 1 May 2009. Each time when traps were monitored, all insects were removed from the traps and then stored at -20° C in a freezer on return to the laboratory. Each trap catch was then sorted and counted in the laboratory, where only the target species, *H. ater, H. ligniperda* and *A. ferus* were recorded and by-catch was discarded.

The role of olfactory and visual cues in host finding were tested in two experiments. Firstly, visual cues in host finding were tested by comparing the effect of different colours

of trap on trap catch of target species, a combination of seven trap types were tested (six colours of panel trap, plus black Lindgren funnel trap). Each trap type was established as a sole trap (see below), (Table 2.1). The combined stimulus of visual and olfactory cues in host finding were tested across the different trap types to compare the visual effect of trap type, to the role of multiple stimulus with the addition of olfactory cues attractant (alpha-pinene and ethanol).

Secondly, host finding behaviours to host and non-host stimuli were tested with four traps treatments of host and non-host visual and olfactory cues. Black traps were used to visually mimic hosts (coniferous tree boles), white to mimic non-hosts (angiosperm tree boles) (Strom & Goyer, 2001; Campbell & Borden, 2005) and clear traps for no visual stimuli. Black, white and clear traps were combined with olfactory stimuli; alpha-pinene and ethanol attractant, green leaf volatile (GLV) repellent, combined alpha-pinene and ethanol with GLV, and control traps without olfactory stimulus (Table 2.1).

Attractant host volatiles used in the experiment consisted of 150 ml alpha-pinene and 150 ml ethanol made up as two separate chemical dispensers described in 2.2.1 (shown in Figure 2.2).

The experimental treatment of non-host volatiles as repellents utilised two green leaf volatile (GLV) compounds, C6-alcohols, that have been found to be dominant constituents of non-host angiosperm leaves and bark (Q. H. Zhang, et al., 1999a), and have shown to repel bark beetles in previous research (Q. H. Zhang, et al., 1999a; Q. H. Zhang, et al., 1999b; Suckling, et al., 2001). The two GLV compounds were (E)-2-hexen-1-ol (Bedoukian, Danbury, USA) and (Z)-3-hexen-1-ol (Bedoukian, Danbury, USA) made up as two separate 20 ml dispensers. Chemical dispensers were made from 200 x 50

mm, 150 μm polyethylene lay-flat tubing with felt strips to assist in even release of
volatiles. When attractant and repellent chemical levels became low they were replaced.

Table 2.1 Trap numbers and treatment type used for the assessment of olfactory and visual cues in host finding of *H. ater, H. ligniperda* and *A. ferus*.

Trap	Alpha−pinene + ethanol	Alpha−pinene + ethanol & GLV	GLV	Control	
Black	10	10	10	10	
Clear	10	10	10	10	
White	10	10	10	10	
Green	10			10	
Red	10			10	
Yellow	10			10	
Funnel trap	10			10	
Total	70	30	30	70	200

Dunedin

To take advantage of the earlier predicted spring flight of *H. ater* (Scion, unpublished report) the olfactory and visual cues in host finding trial was first established in Dunedin at the start of October 2008, before moving the trial to Nelson where there were predicted higher abundance of all three target species (Scion, unpublished report). Five sites were established in third rotation *Pinus radiata* forest in a part of Berwick Forest, approximately 50 km south of Dunedin (Figure 2.3). From 15 to 16 October twenty flight intercept traps were established at each of the five sites, with an experimental design that was the same as described previously for the trial in the Nelson region, trap colour and treatments are described in Table 2.1. Traps were monitored fortnightly until 28 November 2008, when the traps were moved from Berwick Forest to the Nelson region.
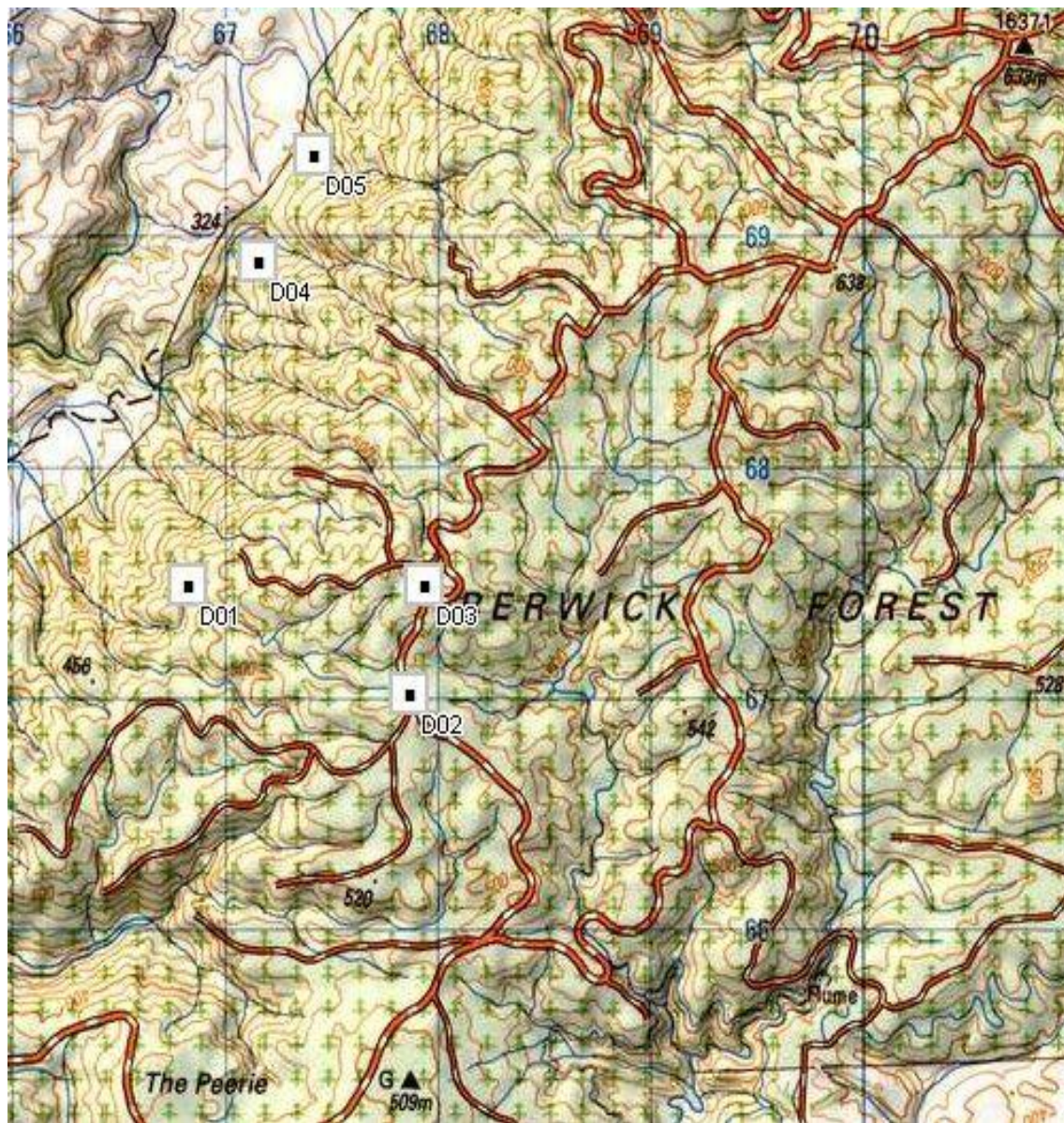
Figure 2.3 Site location (site 1; latitude -45.958; longitude 169.847; altitude 467 m) in Berwick Forest, which is approximately 50 km south-west of Dunedin, Otago, New Zealand. Map extracted from NZMS 1:50,000 series; blue grid lines are 1 km apart.

Data Analysis

Data were analysed by region and by species. I used two main analyses on balanced subsets of the data outlined in Table 2.1: (1) analysis of all trap types and colours, using traps with attractant and control traps with no chemicals; (2) analysis of attractant, repellent, both, and neither on three colours of panel trap (black, white and clear). All analysis was performed on trap catch of each species per trapping day.

The first analysis, effect of visual cues on host-finding through trap type (black, clear, white, funnel trap, green, red, yellow) and treatment (attractant, and control traps with no chemicals) used three Quasipoisson generalized linear models (GLMs), one per insect species, run in R version 2.10.0.

The second analysis, effect of host and non-host visual and olfactory cues on host-finding through olfactory cue (control, attractant, attractant and repellent, and repellent) on three colours of panel trap (black, white and clear) also used Quasipoisson generalized linear models (GLM) in R version 2.10.0.

2.3   Results

2.3.1   Seasonal and Daily Flight Activity

Seasonal Flight Activity

Seasonal flight activity of *H. ater, H. ligniperda* and *A. ferus* were recorded for 7 November 2008 to 1 February 2010 in the forests of the Nelson region (Figure 2.4). Flight activity of *H. ater, H. ligniperda* and *A. ferus* peaked at different times of the year for the different species.

Hylurgus ligniperda was the dominant species during the early spring-summer, with a total of 101,407 recorded in the 20 (later 18) monitoring traps over the 15 months. Monitoring traps were the black panel and black Lindgren funnel traps (Figure 2.2), and trap numbers changed from 20 to 18 with the loss of site N4 during the first field season. *Hylurgus ligniperda* exhibited two peaks of flight activity which is consistent with two generations per year, one in the spring and one in the summer. The peak in activity of *H. ligniperda* was observed to be higher in the spring and summer of the first field season when compared to the second (Figure 2.4), this may relate to an exhaustion of the available host material at the monitoring sites, or the peak summer activity could have been missed when the trapping finished in the summer of the second season.

A total of 2,904 *H. ater* were recorded during monitoring, this species exhibited two peaks of flight activity one in the summer and one in the autumn, staying active through the winter and spring in lower numbers. Peaks in flight activity suggest at least two generations present per year, the numbers observed during the spring were considerably

lower than expected (Scion, unpublished reports); an increased spring flight would be consistent with a suggested third generation (Clark, 1932; Crowhurst, 1969). The numbers of *H. ater* were comparable between the first and second seasons, unlike *H. ligniperda* which was less common in the second season.

Arhopalus ferus exhibited one peak of flight activity per year; adults were only active from November to May, with no activity recorded during the winter. A total of 3,870 *A. ferus* were recorded during the monitoring period. By contrast, *H. ater* and *H. ligniperda* were present in the forests all year round, even during the winter months, although their numbers were considerably reduced (Figure 2.4).

The two trap types used for continued monitoring of flight activity during the project, were commercially available Lindgren 8-funnel trap with the black panel flight intercept traps developed for the trial. The panel trap caught more of the target species over the 15 months monitoring, 42% more *H. ater*, 11% more *H. ligniperda* and 5% more *A. ferus* than the Lindgren funnel trap.
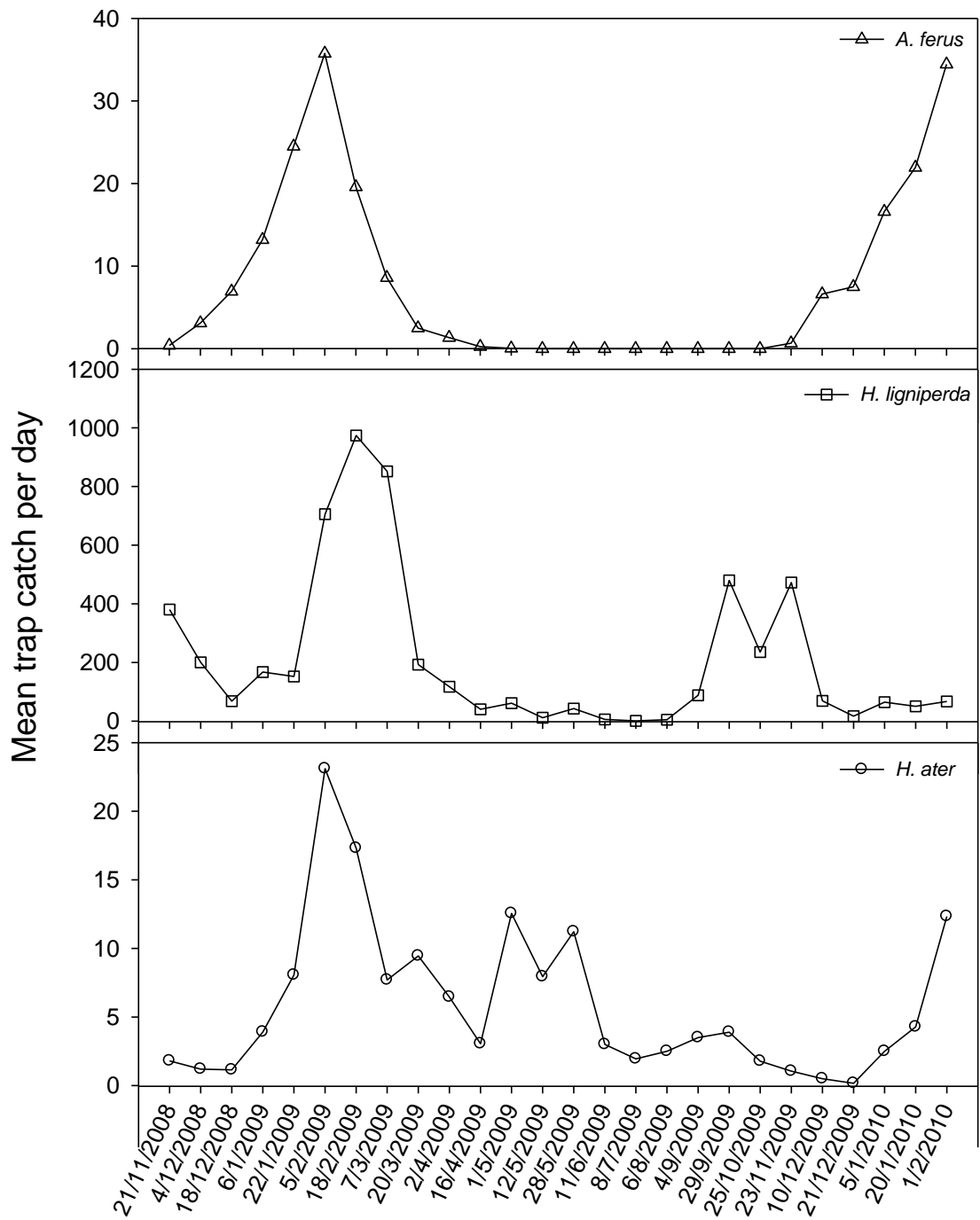
Figure 2.4 Mean number of *H. ater, H. ligniperda* and *A. ferus* individuals caught in flight intercept traps per day over the period November 2008 to February 2010.

Daily Flight Activity

Daily flight activity of *H. ater, H. ligniperda* and *A. ferus* were recorded in February 2009 and January 2010 in the Kainui forest. Daily flight patterns of the combined mean catch over both time periods is presented in Figure 2.5.

Peak flight activity during the day was dominated by *H. ligniperda* which was active during daylight hours, in the morning at 1030 hours, and the evening at 2230 (Figure 2.5). Time of day and year in which the trial was conducted significantly influenced trap catch of *H. ligniperda*, though there was no significant interaction effect of time and year according to a QuasiPoisson generalised linear model (Table 2.2).

Time of the day and the year in which the trial was conducted significantly affected the trap catch of *H. ater*, though there was no significant interaction effect with time of day and year (Table 2.2). *Hylastes ater* exhibits the same pattern to daily flight activity as *H. ligniperda*, showing to be most active at 1030 and 2230, though present in lower numbers during 2009 and not present at all in 2010.

Mean trap catch of *H. ater* and *H. ligniperda* exhibit two peaks of activity during the day, 1030 (for the hours 0730 to 1030), and at 2230 (for the hours between 1930 to 2230) (Figure 2.5). During the summer when the trial was conducted, these time periods incorporate dawn and dusk when the weather is calm, low wind, there is daylight light and a moderate to warm temperature.

The trap catch of *H. ater* and *H. ligniperda* showed a significant effect with year of trial, as there was a considerable reduction in beetle numbers in 2010. There were no *H. ater*

caught during the 2010 trial monitoring daily flight patterns and the number of *H. ligniperda* was reduced by 97%.

Time of day significantly affected the trap catch of *A. ferus* (Table 2.2). The wood boring beetles were most active around dark and into the night, trap catch was recorded the highest at 2230 (1930 to 2230 time period) and 0130 (2230 to 0130 time period) (Figure 2.5).

Table 2.2 Results from generalized linear model with QuasiPoisson distribution testing the effect of time of day and year on the number of *H. ater, H. ligniperda* and *A. ferus* caught per trap.

		Df	Deviance	Resid.Df	Resid.Dev	F	Pr(>F)
Hylastes ater	NULL			45	6.13		
	Time	7	2.58	38	3.56	5.76	**<0.001**
	Year	1	1.52	37	2.03	23.84	**<0.001**
	Time:Year	7	0	30	2.03	0.00	1
Hylurgus ligniperda	NULL			45	270.58		
	Time	7	92.60	38	177.98	4.35	**0.002**
	Year	1	66.70	37	111.28	21.93	**<0.001**
	Time:Year	7	12.29	30	98.99	0.58	0.77
Arhopalus ferus	NULL			45	48.56		
	Time	7	35.96	38	12.61	13.35	**<0.001**
	Year	1	0	37	12.61	0.0013	0.97
	Time:Year	7	0.83	30	11.78	0.31	0.94

*P-values in bold indicate significantly means
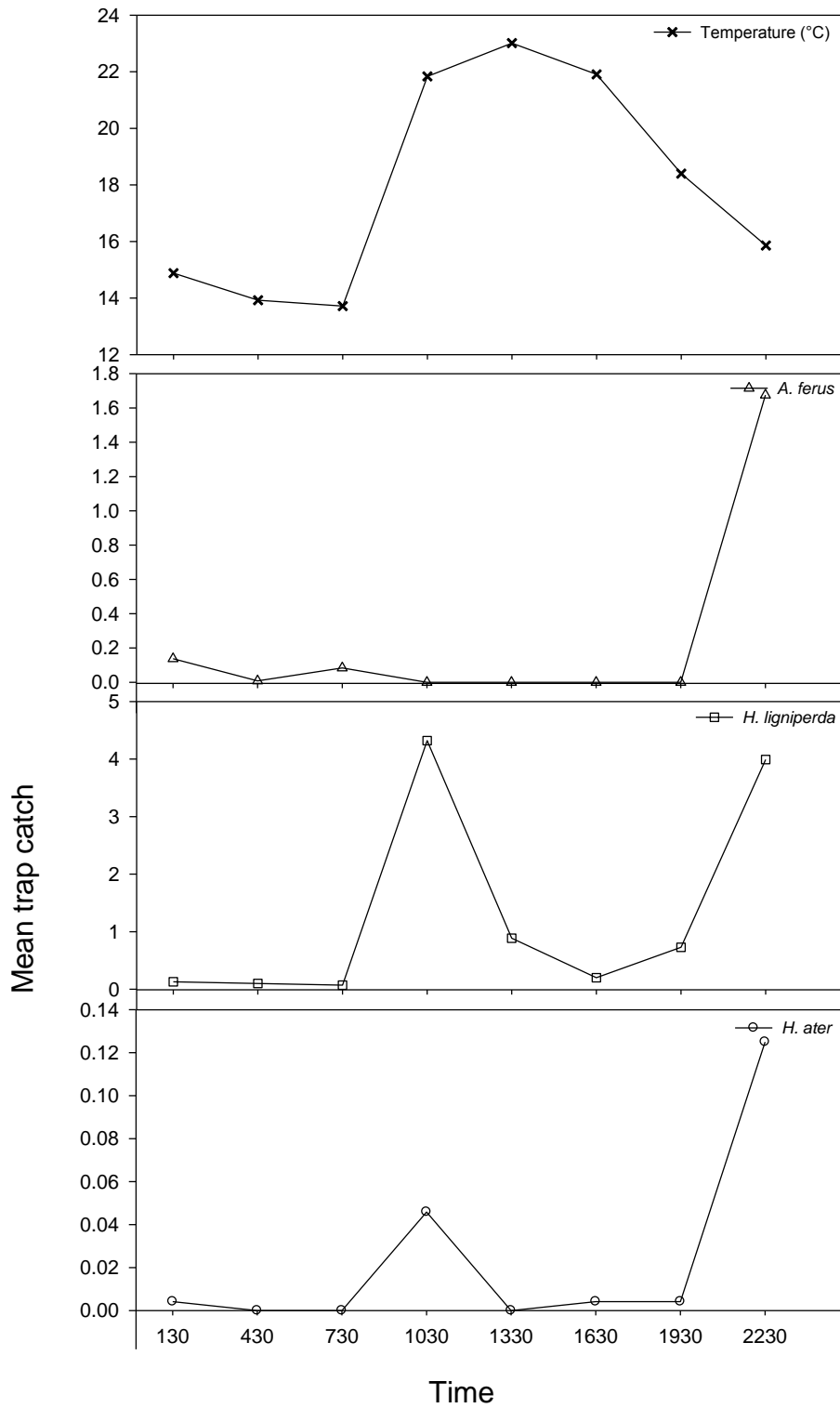
Figure 2.5 Fitted mean catch of *H. ater*, *H. ligniperda* and *A. ferus* and temperature per trap per 3-hour time period. Data are means from two consecutive years, see Methods.

2.3.2   Olfactory and Visual Cues in Host-finding

Nelson

The trial was established during the peak flight activity of *H. ater, H. ligniperda* and *A. ferus* during spring 2008 to autumn 2009 in the Nelson region. Flight activity during this time was dominated by *H. ligniperda*, with a total of 274,594 of this species, plus 7,842 *H. ater* and 16,301 *A. ferus* caught over the 6 months of the trial.

In the first analysis, the influence of visual cues, *H. ater* was significantly affected by the colour of traps, where 'colour' explained 4% of the model deviance (Table 2.3). Red traps were the most attractive, which caught 0.20 *H. ater* per trap, 46% more than the next, the black trap which caught 0.12 beetles per trap (Figure 2.6). Colour of traps significantly influenced the trap catch of *H. ater*; clear traps caught 65% fewer than red traps and had the lowest catch over all traps.

The addition of olfactory cues with 'attractant' on traps explained 42% of the model deviance, the difference between 'sites' significantly influencing trap catch, accounting for 22% of the model. There were no significant interaction effects between 'colour' and 'attractant'. Black traps with attractant caught the highest numbers of *H. ater,* 8 times more than black traps control traps (without attractant). The number of *H. ater* per trap showed a positive, effect with the addition of attractant host volatiles to traps.

Visual cues significantly influenced the trap catch of *H. ligniperda,* where 'colour' explained 3% of model deviance (Table 2.3). Red traps were the most attractive to *H. ligniperda* and caught 0.36 *H. ligniperda* per trap, 54% more than the next best, the black

trap which caught 0.16 beetles per trap. Colour played a small but significant role for host finding of *H. ligniperda*, which were strongly influenced by olfactory cues. This is highlighted by the numbers of beetles caught in clear traps that have no visual stimuli but did not have the lowest catch, an unexpected result. White traps, representing non-host visual cues caught fewer *H. ligniperda* than clear, a 22% difference.

The addition of olfactory stimuli with 'attractant' explained 79% of the model deviance. 'Site' significantly influenced the model explaining 8.5% deviance, and the interaction between 'colour' and 'attractant' explained the rest of the model, though it was not significant. Black traps with attractant caught the highest numbers of *H. ligniperda*, more than 200 times that of the black traps with no attractant (Figure 2.6). Host and non-host specific cues influenced *H. ligniperda* the most, shown by the decrease of 99% from the most influential trap, black with attractant (host mimic) to the least, white (non-host mimic) control trap.

Visual cues significantly influenced the trap catch of *A. ferus* , where 'colour' explained 8% of the model deviance (Table 2.3). The black trap had the highest trap catch with 1.3 beetles per trap, a 6% increase over the red trap which caught 1.2 beetles per trap. Visual cues played a significant role in host finding behaviour of *A. ferus*, where clear traps with no visual stimuli caught the least, 64% fewer beetles than the black traps.

The addition of olfactory cues with 'attractant' explained 4.5% of the model deviance for the number of *A. ferus* caught per trap. Black traps with attractant caught the highest number of *A. ferus* during the trial, approximately 1.4 times more than black traps without attractant (Figure 2.6). Differences between 'site' explained the majority of the model deviance at 72 %, and the interaction between 'colour' and 'attractant' explained the rest

of the model, though there was no significant interaction. The results show both visual and olfactory cues are important for *A. ferus*, the colour or silhouette of the trap is most attractive to *A. ferus,* and attractant, host volatiles have an additive effect on trap catch.

Table 2.3 Results from generalized linear models with QuasiPoisson distribution testing the effect of treatment (site held as block effect); colour/type of traps, and the addition of attractant on the number of *H. ater, H. ligniperda* and *A. ferus* caught per trap per day.

		Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
Hylastes ater	NULL			139	49.02		
	site	9	11.13	130	37.88	9.64	**<0.001**
	colour	6	2.04	124	35.85	2.65	**0.02**
	attractant	1	21.08	123	14.77	164.32	**<0.001**
	colour:attractant	6	0.68	117	14.09	0.89	0.51
Hylurgus ligniperda	NULL			139	2219.37		
	site	9	170.80	130	2048.57	9.68	**<0.001**
	colour	6	66.08	124	1982.49	5.62	**<0.001**
	attractant	1	1762.87	123	219.62	899.59	**<0.001**
	colour:attractant	6	2.00	117	217.62	0.17	0.98
Arhopalus ferus	NULL			139	51.80		
	site	9	37.45	130	14.35	67.12	**<0.001**
	colour	6	4.34	124	10.00	11.68	**<0.001**
	attractant	1	2.32	123	7.68	37.48	**<0.001**
	colour:attractant	6	0.21	117	7.47	0.56	0.76

*P-values in bold indicate significantly means
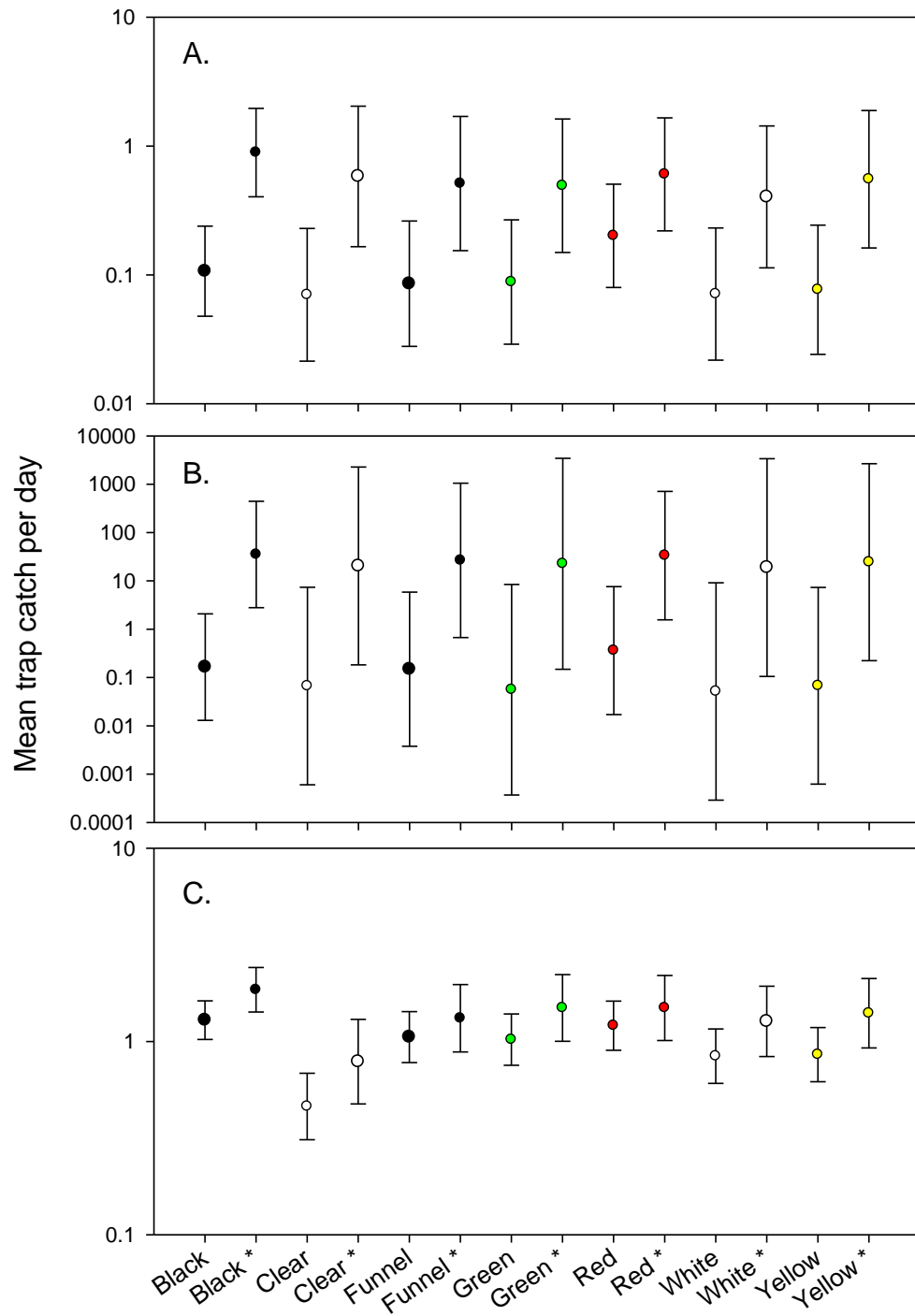
Figure 2.6 Fitted mean number (trap catch per day +/- 95% CI) of (A) *H. ater*, (B) *H. ligniperda* and (C) *A. ferus* per trap treatment; colour with * have attractant (alpha-pinene and ethanol). Y-axis plotted on a log scale. For all three species there were significant effects of trap colour/type and of attractant but no interaction, see Table 2.3.

The second analysis assessed the effect of host and non-host visual and olfactory cues on host-finding through olfactory cue (control, attractant, attractant and repellent, or repellent) on three colours of panel trap (black, white and clear).

Hylastes ater was significantly affected by 'colour', 'attractant' and 'repellent' (Table 2.4). Of the significant effects colour explained 6% of the model deviance, attractant explained 38%, repellent 4%, and, the significant interaction, 'attractant x repellent', 1%. There was no significant interaction between 'colour x attractant', 'colour x repellent', or 'colour x attractant x repellent' (Table 2.4). The combination of repellent (GLV) with attractant (alpha-pinene and ethanol) on traps decreased the number of *H. ater* caught per trap in black, by 95%, white by 81 % and clear traps by 96% when compared to the three trap types with attractant. When repellent GLV was present on traps alone it increased trap catch of black, 29% and clear traps, 48% compared to control traps. The presence of both non-host cues, white traps with repellent GLV decreased trap catch of *H. ater* by 32% over control white traps. The results show, host specific cues, (black trap with attractant) caught more than 10 times *H. ater* that of non-host cues, (white trap with repellent) (Figure 2.7).

Hylurgus ligniperda was influenced in a similar way to *H. ater*, significantly affected by the host and non-host visual and olfactory cues, 'colour', 'attractant' and 'repellent' (Table 2.4) As found in the previous analysis, *H. ligniperda* was strongly and significantly affected by the presence of attractant on traps, black traps with attractant recorded the highest trap catch (Figure 2.7). The results show significant effects of repellent on trap catch, with an average decrease of 90% when repellent was combined with attractant compared to traps with attractant only.

In contrast to the other two species *A. ferus* was only significantly influenced by 'colour' and 'attractant', not 'repellent' (Table 2.4). Black traps with attractant recorded the highest trap catch as found in the previous analysis (Figure 2.7). The trap 'colour' and host volatiles, 'attractant' had the strongest influence on the number of *A. ferus*, shown by the decrease in trap catch of clear traps (which have no visual silhouette) compared to any other trap.

Table 2.4 Results from generalized linear model with QuasiPoisson distribution testing the effect of treatment (site held as block effect); colour of the traps, with the addition of attractant and or repellent, on the number of *H. ater, H. ligniperda* and *A. ferus* caught per trap.

		Df	Deviance	Resid.Df	Resid.Dev	F	Pr(>F)
Hylastes ater	NULL			119	38.905		
	site	9	8.2094	110	30.70	7.22	**<0.001**
	colour	2	2.3941	108	28.30	9.48	**<0.001**
	attractant	1	14.8537	107	13.45	117.59	**<0.001**
	repellent	1	1.3657	106	12.08	10.81	**0.001**
	colour:attractant	2	0.0133	104	12.07	0.05	0.95
	colour:repellent	2	0.0263	102	12.04	0.10	0.90
	attractant:repellent	1	0.4704	101	11.57	3.72	**0.056**
	colour:attractant:repellent	2	0.168	99	11.41	0.67	0.52
Hylurgus ligniperda	NULL			119	1673.72		
	site	9	100.72	110	1573.00	6.31	**<0.001**
	colour	2	110.48	108	1462.52	31.12	**<0.001**
	attractant	1	1251.38	107	211.14	705.10	**<0.001**
	repellent	1	27.52	106	183.62	15.51	**<0.001**
	colour:attractant	2	0.05	104	183.57	0.01	0.99
	colour:repellent	2	14.02	102	169.55	3.95	**0.02**
	attractant:repellent	1	0.08	101	169.47	0.04	0.84
	colour:attractant:repellent	2	0.22	99	169.26	0.06	0.94
Arhopalus ferus	NULL			119	46.079		
	site	9	29.0117	110	17.07	47.51	**<0.001**
	colour	2	8.4559	108	8.61	62.32	**<0.001**
	attractant	1	1.803	107	6.81	26.57	**<0.001**
	repellent	1	0.0166	106	6.79	0.24	0.62
	colour:attractant	2	0.0452	104	6.75	0.33	0.72
	colour:repellent	2	0.0301	102	6.72	0.22	0.80
	attractant:repellent	1	0.0453	101	6.67	0.67	0.42
	colour:attractant:repellent	2	0.0275	99	6.64	0.20	0.82

*P-values in bold indicate significantly different means
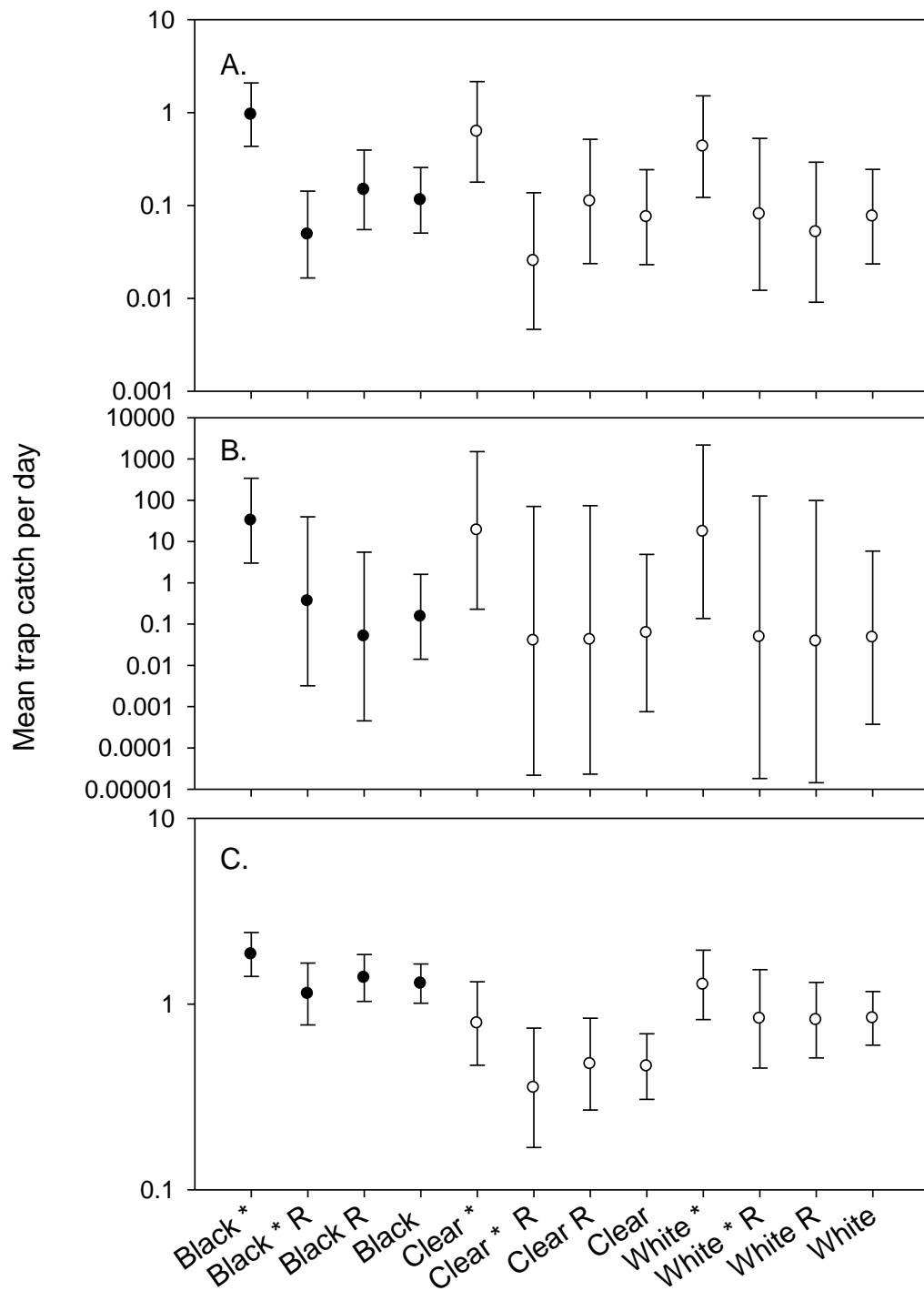
Figure 2.7 Fitted mean number (trap catch per day +/- 95% CI) of (A) *H. ater*, (B) *H. ligniperda* and (C) *A. ferus* with trap treatment; colour of traps (Black, White or Clear) where colour alone is control, with attractant (*), attractant with repellent (* R) and repellent (R). Y-axis plotted on a log scale.

Dunedin

Seasonal flight activity of *H. ater* and *H. ligniperda* was recorded in October-November 2008 in Berwick Forest (Figure 2.8). In total the catch of *H. ater* (3,396 beetles) was much higher over the six weeks, than *H. ligniperda* (63 beetles), which did not increase until the last two weeks of the trial period. Due to the low numbers of *H. ligniperda* this species was not included in data analysis.
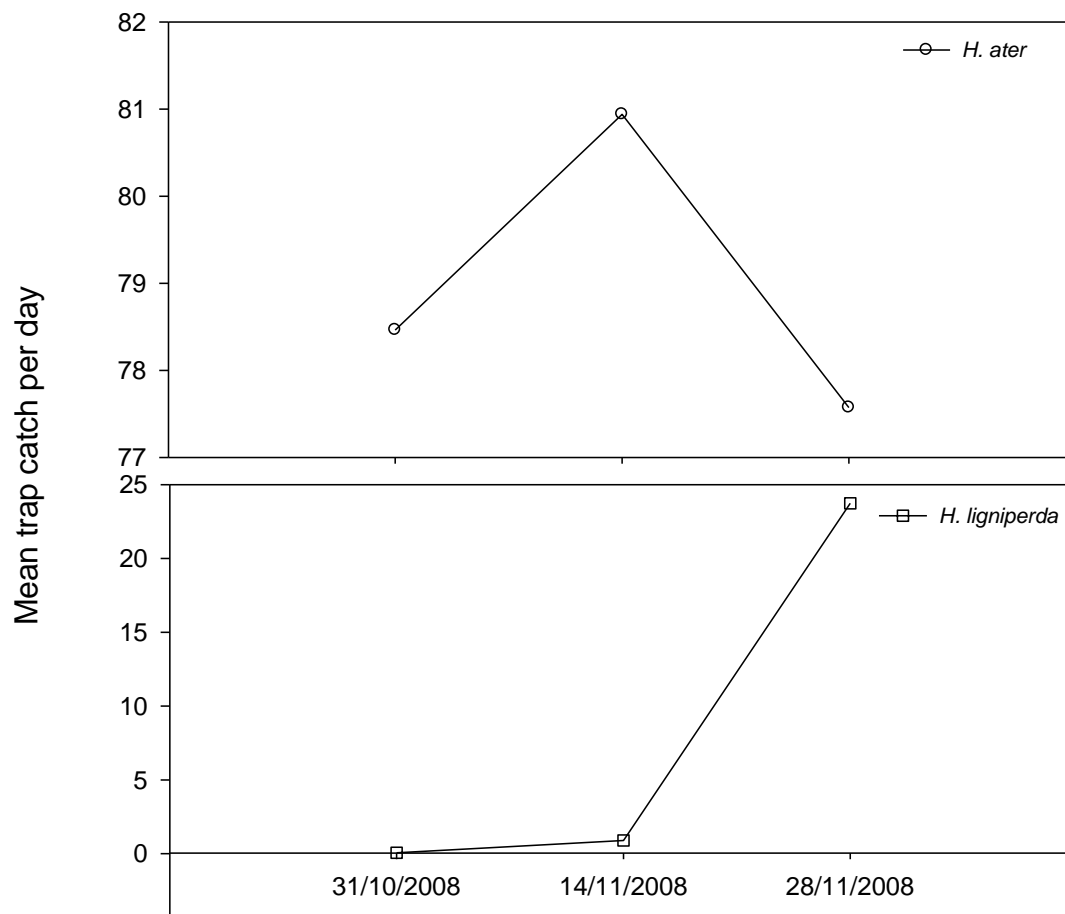
Figure 2.8 Mean trap catch per day of *H. ater* and *H. ligniperda* from Berwick Forest, Dunedin.

H. ater showed the same trap preferences in Dunedin as in Nelson, and was significantly affected by the colour of traps, where 'colour' explained 15% of the model deviance (Table 2.3). Funnel traps were the most attractive, with 0.19 *H. ater* per trap per day, 18% more than the next, the red trap (0.16 per trap), and 34% more than the black panel trap (0.14) (Figure 2.9). Colour of traps significantly influenced the trap catch of *H. ater*; clear traps caught 76% fewer than funnel traps, similar to green traps which had the lowest catch over all traps. The addition of olfactory cues with 'attractant' on traps explained 56% of the model deviance, the difference between 'sites' significantly influencing trap catch, accounting for 6% of the model. There were no significant interaction effects between 'colour' and 'attractant'. Over all traps, red traps with attractant caught the highest numbers of *H. ater,* more than 10 times the catch of red control traps (without attractant). Black traps with attractant were the next most attractive combination, 1.51 *H. ater* per trap per trapping day, 20 % fewer than red with attractant.

The second analysis assessed the effect of olfactory cue (control, attractant, attractant and repellent, and repellent) and colours of panel trap (black, white and clear). As in the previous analysis 'colour' and 'attractant' had a significant effect on trap catch of *H. ater* (Table 2.6). And as expected black traps recorded the highest trap catch (Figure 2.10). In contrast to results presented from Nelson, repellent had no significant effect on trap catch of *H. ater.*

Table 2.5 Results from generalized linear model with QuasiPoisson distribution testing the effects of site (held as block effect); colour/type of traps and attractant on the number of *H. ater* caught per trap m Dunedin.

	Df	Deviance	Resid.Df	Resid.Dev	F	Pr(>F)
NULL			69	81.28		
site	4	4.54	65	76.74	3.59	**0.01**
colour	6	12.95	59	63.79	6.83	**<0.001**
attractant	1	46.13	58	17.67	145.99	**<0.001**
colour:attractant	6	1.37	52	16.30	0.72	0.63

P-values in bold indicate significantly different means
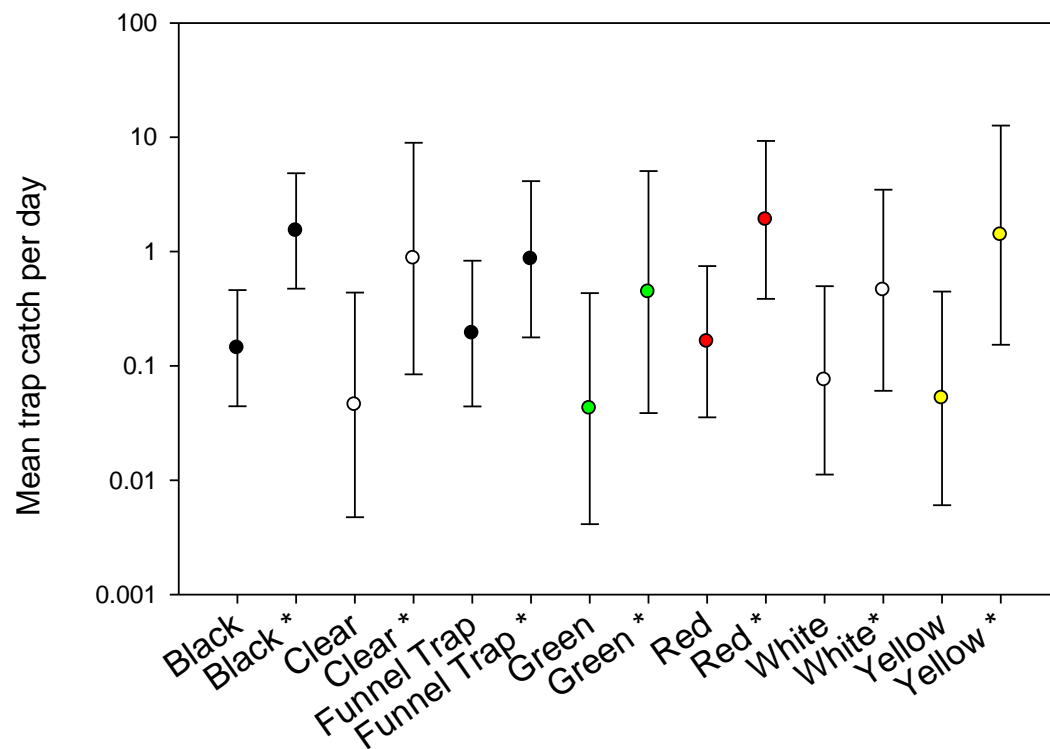
Figure 2.9 Fitted mean number (trap catch per day +/- 95% CI) of *H. ater* caught per trap treatment; colour with * have attractant. Y-axis plotted on a log scale.

Table 2.6 Results from generalized linear model with QuasiPoisson distribution testing the effects of treatment (site held as block effect); colour of the traps, with the addition of attractant and or repellent on the number of *H. ater* caught per trap.

|	Df	Deviance	Resid.Df	Resid.Dev	F	Pr(>F)
NULL			59	63.24		
site	4	7.64	55	55.60	5.68	**<0.001**
colour	2	9.56	53	46.05	14.22	**<0.001**
attractant	1	28.86	52	17.19	85.90	**<0.001**
repellent	1	0.05	51	17.14	0.14	0.71
colour:attractant	2	0.28	49	16.86	0.41	0.67
colour:repellent	2	0.72	47	16.14	1.07	0.35
attractant:repellent	1	0.50	46	15.65	1.48	0.23
colour:attractant:repellent	2	0.97	44	14.68	1.44	0.25

*P-values in bold indicate significantly different means
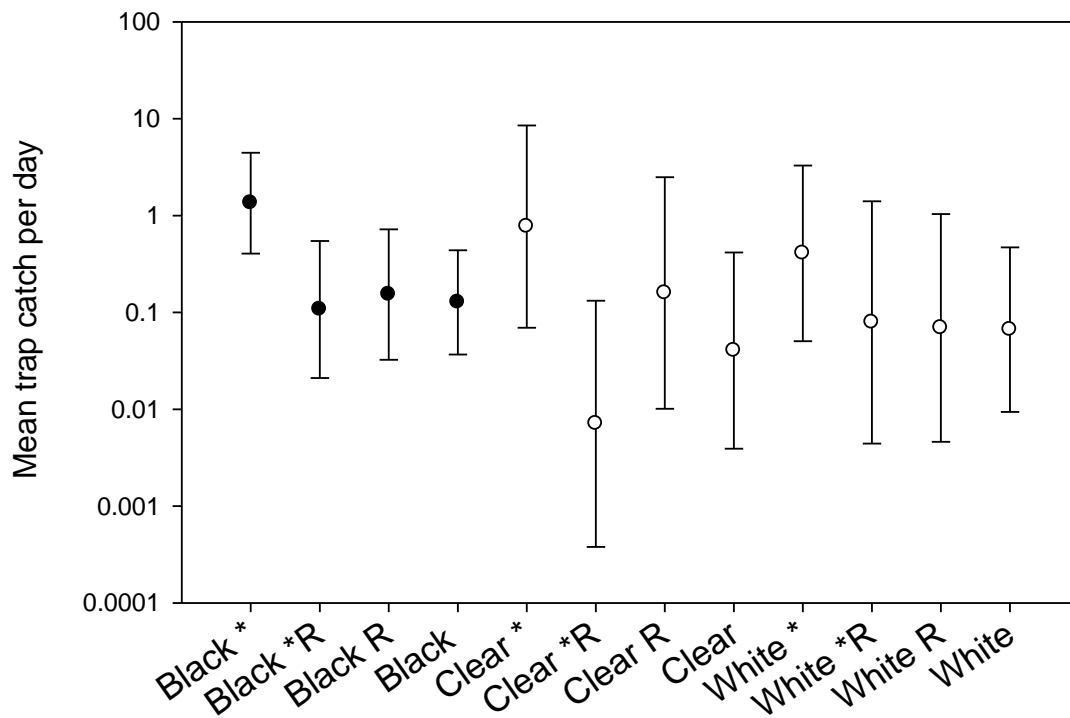
Figure 2.10 Fitted mean number (trap catch per day +/- 95% CI) of H. ater with trap treatment; colour of traps (Black, White or Clear) where, colour alone is control, with attractant (*), attractant with repellent (* R) and repellent (R). Y-axis plotted on a log scale.

2.4   Discussion

2.4.1   Daily and Seasonal Flight Activity

Daily activity differed between species over the trial period; *H. ater* and *H. ligniperda* were most active during dawn and dusk in the summer. *Arhopalus ferus* was found to be active from dusk through the night, supporting results from laboratory experiments that showed predominant nocturnal activity (Suckling, et al., 2001).

Over both trial periods when a decrease in temperature occurred there was also a decrease in the number of beetles caught. Reay & Walsh (2001) found an association with flight activity of *H. ater* and *H. ligniperda* and atmospheric pressure and Clark (1932) described *H. ater* as a strong flyer in the sunlight. Seasonal weather conditions have an effect on the flight activity, once emerged adult beetles are most active in calm light filled environmental conditions that would support the use of visual cues during flight through a strong olfactory landscape. There needs to be more research into the factors that influence daily flight activity of *H. ater* and *H. ligniperda* to better predict the movements of beetles within forestry.

Activity of *H. ater, H. ligniperda* and *A. ferus* was dominated by peaks of activity separated by different times of the year, even though they are found to occupy the same habitat (Reay, 2001; Brockerhoff, et al., 2006b), the majority of beetles were caught between late November and May.

The trap catch of *H. ater* in Nelson demonstrates two distinct peaks of flight activity or bimodal flight activity (January/February and May). Previous research has found

contradicting results with early observations by Crowhurst (1969) supporting a bimodal pattern to flight activity from overlapping generations. Research after the introduction of *H. ligniperda,* in the North Island of New Zealand found only a single peak of activity during the autumn suggested to be due to competition with *H. ligniperda* (Reay & Walsh, 2001). My results thus support reports of two peaks of flight activity, for two or potentially three overlapping generations depending on the small peak in activity in the spring for *H. ater*.

Flight activity during the summer months was dominated by large numbers of *H. ligniperda* which also show bimodal flight activity but starting earlier in the year than *H. ater*, in the spring and again in summer. Similar results but slightly earlier than previous research in the North Island which found *H. ligniperda* to be bimodal with two peaks of activity during the summer (Reay & Walsh, 2001). *Hylurgus ligniperda* in Chile shows a similar distribution in flight activity which Mausel et al (2007) suggested could be due to overlapping generations, and in Chile *H. ligniperda* dominate in numbers during peaks of flight activity over those of *H. ater* (Ciesla, 1988). In South Africa *H. ligniperda* shares a similar activity pattern in its interaction with *Hylastes angustatus*, and is dominant over the summer period (Tribe, 1991). In New Zealand *H. ater* and *H. ligniperda* adults were active throughout the year, though minimal flight activity was observed during the winter, a pattern that has been previously described by Reay & Walsh (2001) in New Zealand, Mausel et al (2007) in Chile, and Tribe (1991) in South Africa.

Arhopalus ferus adult flight activity in the forests of Nelson was restricted to November through May, with no adults found outside these months during the year, consistent with a lifecycle of 1 year producing one generation per year (Brockerhoff & Hosking, 2001).

The results were generally consistent with reports previously published on seasonality and peaks in flight activity of *H. ater, H. ligniperda* and *A. ferus* in New Zealand (Clark, 1932; Crowhurst, 1969; Brockerhoff & Hosking, 2001; Reay & Walsh, 2001).

Research monitoring bark beetle species has predominately utilised one type of flight intercept trap, the Lindgren multiple funnel trap and this has become broadly accepted as an industry standard, for example - (Strom, et al., 1999; Borden, et al., 2001; Reay & Walsh, 2001; Strom & Goyer, 2001; Huber & Borden, 2003; Brockerhoff, et al., 2006b; Campbell & Borden, 2006b; Hayes, et al., 2008; Miller & Crowe, 2009; Miller & Rabaglia, 2009). The current research tested the efficiency of the Lindgren 8-funnel flight intercept trap against the panel flight intercept trap. Overall the black panel trap caught higher numbers of all target species required for this research project. Therefore this trap design appears to be more effective in trapping the target species in the forests around New Zealand.

2.4.2   Olfactory and Visual Cues in Host-finding

Generally, coniferous wood-boring and bark beetle species are attracted to host specific volatiles (D. L. Wood, 1982), and will avoid volatile semio-chemicals that are predominately found within non-host angiosperm trees, specifically leaves and bark, reviewed in - (Q. H. Zhang & Schlyter, 2004). Secondary bark beetle species, generally colonise harvested, fallen, or wind thrown plant material that is generally aged and are known to utilise host specific cues in foraging behaviour (Allison, et al., 2004). Bark beetle species may combine specific cues in host finding such as visual stimulus accompanied by olfactory stimulus from hosts and non-hosts in multi-modal host finding behaviour (Campbell & Borden, 2009). Few have tested multi-modal cues that incorporate both host and non-host recognition (Campbell & Borden, 2006a, 2006b, 2009). The influence of olfactory and visual cues in host finding of *H. ater, H. ligniperda* and *A. ferus* was assessed through colour, attractant and repellent in a two stages. *Hylastes ater, H. ligniperda* and *A. ferus* are attracted to pine specific host volatiles, alpha-pinene and ethanol, consistent with previous research with these species and other bark beetle species (Schroeder & Lindelöw, 1989; Byers, et al., 1998; Reay, 2001; Suckling, et al., 2001; Reay & Walsh, 2002c; Byers, 2004; Brockerhoff, et al., 2006b).

The influence of colour in host finding decisions found that all three species are either attracted to darker coloured traps in red or black (host stimuli) over white (non-host stimuli) or clear (no-visual stimuli). Traps tested colours including host and non-host mimics at either end of the known wave length spectrum of bark beetle vision, between UV and red (350 nm – 650 nm) (Prokopy & Owens, 1983). This suggests the beetles were

not being caught at random but were making decisions on where to land according to the visual stimuli received. Even though host specific (black) traps were preferred to non-host (white) traps there were still beetles caught in white traps, indicating that some were caught through choosing to land or accidental interception. The addition of attractant host volatiles increased the trap catch of all three species, suggesting there maybe integrated visual and olfactory information when host finding.

For wood-boring and bark beetles the difference in colour or reflectance could complement the odours used to discriminate between hosts and non-hosts (Campbell & Borden, 2005). In support of this hypothesis results from previous research found coniferous bark beetles avoided white and black attractant baited traps (Strom & Goyer, 2001; Strom, et al., 2001; Campbell & Borden, 2006b).

The current research found that the primary attraction for *H. ater, H. ligniperda* was to host volatiles over the colour of the trap. Attractant increased trap catch between 1 and over 100 times more than control traps with no attractant, depending on species. Though *A. ferus* was more strongly influenced by colour, than host volatiles, they did show an additive effect, increasing attraction to traps to all except clear with attractant. Suggesting there may also be redundancy in processing multiple stimuli. All three beetle species seem to orientate to host volatiles, accepting appropriate visual stimuli associated with volatiles, then decide to land. This is supported by larger catch with host volatiles and host specific black traps over clear and white. Suggesting that host volatile presence is a dominant factor in initiating host finding behaviour, with the influence of visual cues in close range aiding to distinguish hosts from non-hosts (Campbell & Borden, 2006a).

Close range acceptance of visual cues is consistent with limited vision of insects (Prokopy & Owens, 1983), where they would have to be close to an object to distinguish visual stimuli. This physiological restraint supports why the results do not show a solid one trap colour preference. This effect is consistent with previous research into host finding by bark beetle species which found increased attraction to different traps types with the addition of host stimuli (Schroeder & Lindelöw, 1989; Reay & Walsh, 2002c; Q. H. Zhang & Schlyter, 2003; Campbell & Borden, 2009). It is understood that host specific species of bark beetle such as *H. ater, H. ligniperda* and *A. ferus* prioritise olfactory cues in host finding with the capability of using visual cues in foraging when presented with host specific information (Campbell & Borden, 2006a), and more generalist species may not require this combination from behavioural cues as they have strong responses to each mode of host finding behaviour individually (Campbell & Borden, 2006b).

To expand on the influence of host and non-host cues in host finding behaviour, visual stimulus of black, white and clear traps was combined with olfactory stimulus from attractant and repellent volatiles. The application of green leaf volatile (GLV), repellent generally reduced trap catch irrespective of colour. The addition of GLV to non-host (white) traps showed the greatest reduction in the catch of *H. ater* and *H. ligniperda* of 95-99% compared to host specific traps, black with attractant host volatiles which had the highest trap catch.

Though *A. ferus* did not show a significant effect to repellent within the results there was reduced attraction to white traps more so than black traps with GLV repellent. Results for *A. ferus* were consistent with previous research in laboratory experiments from Suckling

et al (2001) who found green leaf volatiles to deter *A. ferus* from oviposition on host material treated with green leaf volatiles in emulsion.

All three species show a strong response host specific cues, where visual cues elicit a behavioural response in the presence of the appropriate or inappropriate olfactory stimuli for host selection behaviour. Olfaction drives *H. ater* and *H. ligniperda* more so than *A. ferus* who had a greater response colour or silhouette of traps. Darker trap colours were more attractive than light or no visual stimulus. This response is consistent with the daily activity of this species, which is essentially nocturnal. Dark silhouettes of traps would be of greater visual significance to a rudimentary insect eye over light or no visual stimulus even with host volatiles.

Having the ability to utilise multiple host finding cues may aid in defining visual and olfactory sensory overlap experienced when presented with a natural forest environment, with many non-host species that have to be negotiated in order to find a suitable host. Consistent with the knowledge that foragers should combine information across sensory modes to increase the individual fitness in host finding behaviour (Strom, et al., 1999; Campbell & Borden, 2009). Avoidance of combined visual and olfactory non-host cues is generally in an additive fashion (Campbell & Borden, 2009), even though all three species were present in every trap type, which may indicate a high instance of chance intercept, the addition of non-host cues decreased their number caught below that of host specific cues consistent with an additive response.

The results are consistent with multi-modal olfactory and visual cue use in host finding behaviour that would aid in finding host material in a natural forest environment that can be widely distributed. The ability of *H. ater, H. ligniperda* and *A. ferus* to successfully

discriminate between olfactory and visual host and non-host cues enables them to become predominant pests in plantation forestry where host material is readily available with little presence from non-host species to limit host finding abilities and population growth.

3   THE EFFECT OF NON-HOST LEAF VOLATILES

AS REPELLENTS TO *HYLASTES ATER* AND

HYLURGUS LIGNIPERDA

3.1   Introduction

Wood-borers and bark beetles can cause a variety of damage to host trees and wood products. Their brood galleries and feeding tunnels under the bark and in the wood of felled and standing trees and can damage and degrade the wood, introducing sap staining and decay fungi to the natural resources they inhabit (D. L. Wood, 1982; S. L. Wood, 1982; Reay, 2001; Sauvard, 2004; Leahy, et al., 2007; Mausel, et al., 2007; Brownbridge, et al., 2010). Such beetles can also be important quarantine pest if they are found in timber or wooden products destined for export (Brockerhoff & Hosking, 2001; Z. Zhang, et al., 2004; Zahid, et al., 2008)

In natural forest environments such an abundance of woody debris would not normally be available, more or less continuously. Introduced bark beetle species, can reach epidemic levels because of the lack of specific natural enemies or host defences which may limit their population growth (Colautti, et al., 2004). For this reason, there are increasingly strict quarantine regulations on the export of logs and timber, which require the use of various treatments such as fumigation or heat treatment to reduce the risk of further biological invasions (Z. Zhang, et al., 2004). However, there are concerns that the use of methyl bromide and other fumigants as quarantine treatments pose a human health hazard, and the use of such treatments is becoming socially unacceptable (Lanfranco, et

al., 2004; Z. Zhang, et al., 2004). Furthermore, quarantine measures by themselves do not solve the problem of pests within the environment. If pest numbers could be reduced at the source, within forests, then the need to use chemical treatments as phytosanitary measures could decrease within the export sector.

New Zealand has substantial areas of plantation forests, approximately 1.8 million hectares (Annon, 2010) predominantly stocked with *Pinus radiata*. When stands of forest are harvested it creates a plentiful supply of woody debris which serves as host material for wood borers and bark beetles. Cultural methods that were used historically for the control or prevention of bark beetle damage in forests and on export timber employed the monitoring of plant stock health, uprooting of stumps, burning of infested plants and debris, aerial and ground application of chemical pesticides to logs, lumber, and newly planted seedlings, along with rapid removal and turnover of timber stocks (Dowding, 1973; Milligan, 1978; Zondag, 1982; Borden, et al., 2001). In New Zealand forestry, practices have been updated with cleaner cultural methods, with good cultivation, plant hygiene, removal and rapid turnover of timber, but these methods have not been enough to control the growing pest problem. This has necessitated the investigation into further control techniques including the use of biological control agents (Milligan, 1978; Zondag, 1982; Faulds, 1989), resistant plant strains (Reay, 2001), the environmentally more responsible use of pesticides and non ozone-depleting fumigants (Allan & Higgs, 2000; Allan, et al., 2000; Reay & Walsh, 2002a; Rolando & Allan, 2004; Z. Zhang, et al., 2004; Rolando, 2006; Leahy, et al., 2007). Furthermore, it has been suggested that the implementation of mixed planting strategies may be beneficial by reducing risks of insect outbreaks potentially associated with the cultivation of 'monocultures' (Jactel, et al., 2005; Jactel & Brockerhoff, 2007).

The bark beetles, *H. ater* and *H. ligniperda* are active in New Zealand forests during much of spring, summer and autumn (Chapter 2). Hence, harvesting of trees at most times of the year will generate harvesting slash that can be attacked by beetles. Population numbers are expected to be closely related to the abundance of available host material. Therefore, there is a high risk of bark beetles colonising harvested timber, especially if it is in contact with soil and out in open cut over areas (Mausel, et al., 2007). Beetle attack also contributes to the inoculation of timber with sapstain and decay fungi (Dowding, 1973; Suckling, et al., 1999; Reay, et al., 2002; Brownbridge, et al., 2010) if it is not promptly removed from harvested stands. The presence and abundance of bark beetles, including *H. ater* and *H. ligniperda*, have been related the colonisation of logs and the spread of sapstaining fungi in New Zealand (Suckling, et al., 1999; Brockerhoff & Bain, 2000; Reay, 2001; Reay & Walsh, 2002b; Reay, et al., 2002; Brownbridge, et al., 2010), and Chile (Ciesla, 1988; Lanfranco, et al., 2004; Mausel, et al., 2007).

Hylastes ater is known to attack and damage conifer seedlings in New Zealand (Clark, 1932; Zondag, 1965; Crowhurst, 1969; Milligan, 1978; Zondag, 1982; Reay & Walsh, 2002b, 2002a; Reay, et al., 2002), and around the world (Milligan, 1978; Lindelöw, 1992, 1992a.; Leahy, et al., 2007), resulting in feeding damage to the root collar and often girdling the pine seedlings. Changes in forestry practices with increased demand on forest production have increased the abundance of *H. ater* and other bark beetle species within forestry (Leather, et al., 1999; Orlander & Nilsson, 1999), mainly from a decreased fallow time after harvest. However, assessments about the role of *H. ater* in the re-establishment of stands and the extent of damage to seedlings has been controversial (Zondag, 1965, 1968; Milligan, 1978; Lindelöw, 1992, 1992a.; Reay, 2001; Leahy, et al., 2007) due to misdiagnosis of the cause of seedling death and inconsistent results in assigning cause of

death of seedling plants. Mortality is generally low but even low level of loss could necessitate costly re-planting to ensure even stand growth (Reay & Walsh, 2002a). Recent seedling trials in the North Island reported seedling mortality of only 5% due to *H. ater* (Reay & Walsh, 2002b), but there have been earlier reports of up to 50% mortality (Reay & Walsh, 2001), and as high as 90% in Australia (Boomsma & Adams, 1943), and 70% in Chile (Ciesla, 1988). Also, even if attacks do not result in mortality, high levels of sub-lethal attack may be observed. The full effect of sub-lethal attack is unclear, however, it has been reported that there is a strong positive relationship between increased attack by *H. ater* on *P. radiata* seedlings and the presence of sapstain fungi within seedling trees (Reay, et al., 2002; Reay, et al., 2005; Brownbridge, et al., 2010). The invasion of fungi into sub-lethally damaged seedlings could potentially impact on the tree health later on and therefore reduce forest health (Reay, et al., 2005). Treatments for the control of *H. ater* in regeneration have concentrated on the use of pesticides. In New Zealand, South Africa, Chile and Europe this has proven to decrease the risk from *H. ater* and other *Hylastes* species which attack seedlings without having to leave areas of forest fallow for long periods of time after harvest (Dowding, 1973; Ciesla, 1988; Lindelöw, 1992; Leather, et al., 1999; Allan & Higgs, 2000; Allan, et al., 2000; Reay & Walsh, 2002a; Rolando & Allan, 2004; Rolando, 2006). However, even though the use of pesticides has proven effective in control, the cost of chemicals and their application restricts their operational use. Therefore, other management techniques are required.

The ability to find suitable host breeding material becomes the limiting factor of all bark beetles (Lindelöw, et al., 1992; Knizek & Beaver, 2004). Adults emerge from host material and disperse to find new areas to colonise. Disrupting this host-finding process may be one way of limiting the reproductive success of bark beetles, thereby reducing

their populations. Control options within forestry aim to focus at the source of the problem, targeting bark beetles with the use of non-host cues to disrupt location of host material (Borden, et al., 2001; Suckling, et al., 2001; Goyer, et al., 2004). During host location, the majority of bark beetles orientate by using volatile chemicals emitted from host material as cues (D. L. Wood, 1982; Schroeder & Lindelöw, 1989; Brockerhoff, et al., 2006b; Seybold, et al., 2006). Non-host cues may disrupt this behaviour and disorientate beetles which can reduce their ability to find their host, and this can be exploited for the protection of trees and wood products (Schroeder, 1992; Borden, et al., 2001; Huber & Borden, 2001b; Suckling, et al., 2001; Q. H. Zhang & Schlyter, 2003; Byers, et al., 2004; Goyer, et al., 2004; Campbell & Borden, 2006b, 2009). The effects of non-host cues have also been related to theory about the functional significance of biodiversity in forest ecosystems. It has been argued that the complex visual and olfactory 'landscape' in mixed forests reduces the risks from outbreaks of pests and diseases, compared with less diverse forests (Prokopy & Owens, 1983; Jactel, et al., 2005; Jactel & Brockerhoff, 2007).

Non-host olfactory and visual cues from angiosperm trees have been shown to decrease the numbers of the secondary bark beetles *H. ater* and *H. ligniperda* attracted to host mimicking monitoring traps (Chapter 2). Although the use of monitoring traps is useful for testing host and non-host volatiles in trapping trials, it is important to determine whether treatments based on these volatiles can be used to reduce attack of cut logs and timber stocks in areas such as ports and mills. The use of non-host volatiles such as green leaf volatiles (GLVs) has the potential to reduce the reliance on toxic chemical pesticides and may contribute to the implementation of environmentally more acceptable ways of managing bark beetles.

The effects of GLVs on attack of *H. ater* and *H. ligniperda* in New Zealand forests were tested in two ways, with the following objectives:

- Firstly, to determine the effect of non-host volatiles on attack of *P. radiata* logs, I tested the effects of topical applications of GLVs and of naturally emitted volatiles from angiosperm plants growing among pine trees.

- Secondly, to determine the effect of non-host volatiles on attack and mortality of *P. radiata* seedlings in Dunedin, I tested several GLV formulations in pot and field trials.

3.2   Methods

3.2.1   Effects of Non-host Volatiles on Attack by *Hylastes ater* and *Hylurgus ligniperda* of *Pinus radiata* Logs

The effects of non-host volatiles (green leaf volatiles) on attack by *H. ater* and *H. ligniperda* of *Pinus radiata* trap logs was assessed in two experiments, by topical application and by using naturally emitted non-host volatiles from broadleaved plants. Attack by the bark beetles was quantified by counting adults present under the bark of freshly cut *P. radiata* trap logs.

On 23 September 2009, trial sites were selected from a small area of second rotation *P. radiata* forest in Selwyn Plantation Board's Chaneys Forest, NE Christchurch. With the assistance of a Selwyn Plantation Board forester, trees of the same age and with similar bark characteristics were selected and cut into 100 trap logs measuring 0.5 m long by approximately 0.20 m diameter.

The trap logs were placed in the field during the spring flight of *H. ater* and *H. ligniperda*. At trial establishment, two Lindgren 8-funnel flight intercept traps (Phero Tech Inc., Delta, British Columbia, Canada) were installed, suspended on wire from 1.6 m steel posts at each end of a recently clearfelled site that was selected to assess topically applied non-host volatiles. These traps monitored the flight activity of *H. ater* and *H. ligniperda* during the spring and into summer, to ensure the logs were in the field while beetles were active. Each week the traps were cleared and numbers of beetles caught per trap recorded.

Topically applied non-host volatiles

To assess the effects of topically applied GLVs on attack by *H. ater* and *H. ligniperda* of *P. radiata* trap logs, one site was selected in a large easily accessible area of Chaneys Forest. The site had been felled in 2009 and contained a large amount of fresh logging debris conducive to the presence of bark beetles. Fifty 0.5 m long trap logs were set out in five replicate lines of ten logs across the site. Logs were placed at a distance of 20 m apart with replicate lines at least 50 m away. Due to the site characteristics the lines ran between and parallel to wind rows of logging debris. Each trap log was placed in an east - west direction to standardise the direction of the logs with respect to insolation such that one long side was exposed and the other shaded. Experimental treatment of non-host volatiles applied green leaf volatiles (GLV) in solution with carrier oil applied to every second log within each replicate, the remaining logs were left as natural (control) trap logs.

The green leaf volatile (GLV) used in this experiment was the same chemical used in the previous trapping trial (Chapter 2). GLV consisted of both green leaf alcohols (E)-2-hexen-1-ol (Bedoukian, Danbury, USA) and (Z)-3-hexen-1-ol (Bedoukian, Danbury, USA), used in a mixture with silicon oil as carrier (70% oil to 30% GLV). The treatment was applied with a paint brush at 50 ml (15 ml GLV) per treated log, covering the logs entirely with solution.

The presence of beetles under the bark of trap logs was assessed by counting the number of characteristic bore holes made by the bark beetles through the bark. The holes are made by the beetles to reach the cambium and phloem region where they feed and breed. The bore holes were marked with small, plastic round topped mapping pins on a weekly basis.

Naturally emitted non-host volatiles

To assess the effects of naturally emitted non-host volatiles on attack of *P. radiata* logs by *H. ater* and *H. ligniperda*, five sites in Chaneys Forest were selected that all contained areas of dense understorey vegetation of broadleaved shrubs as well as clear, open areas with no understorey vegetation. The dense vegetation available varied among sites, with different proportions of broadleaved 'weeds', including Scotch broom (*Cytisus scoparius* (L.)), gorse (*Ulex europaeus* (L.)) or bush lupin (*Lupinus arboreus* (S.)). These species, all of which are members of the legume family (Fabaceae), and emit natural volatiles from leaves and bark that would be considered non-host volatiles from the point of view of bark beetles which attack pines. Fifty 0.5 m-long trap logs were utilised across the five selected sites. At each replicate site logs were set out in five pairs of two logs, one log in the open and one nearby at least 5 m away under the available broad leaved vegetation. Pairs were placed with the available open and vegetation areas per site, no less than 50 m apart. Logs were checked on a weekly basis for beetle attack as in the first experiment testing the influence of topically applied non-host volatiles (see above).

Over the period of this trial it sustained the loss of six out of the 50 trap logs. These were apparently removed from the trial sites by people frequenting the forest. Four logs from the fifth site and one log from both the first and second sites were lost, representing four from open sites and two from broadleaved vegetation. Because of this the experimental design became unbalanced, which was accounted for in the data analysis.

Assessment of trap logs

All trap logs were removed from the field and taken to the laboratory on 16 November 2009. At that time each log was individually bagged and placed in a freezer at -20° C to stop beetle activity, tunnel development and gallery formation.

Laboratory analysis to assess the extent of *H. ater* and *H. ligniperda* attack on trap logs for both experiments was carried out with the help of two research assistants. The bark was removed with a knife and chisel from each trap log in order to count the number of adult beetles present. Due to the relatively short time the logs were in the field, only colonising adult beetles were found under the bark of the logs (i.e., no adults from the next generation were present yet).

Data analysis

The effects of topically applied GLVs on the number of *H. ater* and *H. ligniperda* that attacked trap logs were investigated through a Quasipoisson generalized linear model (GLM). Effects of naturally emitted non-host volatiles on bark beetle attack of trap logs were investigated through a Quasipoisson generalized linear mixed model (GLMM) because the unbalance nature of this experimental design (due to the loss of some logs) precluded the use of GLM. The GLMM output does not directly test for a location effect, but includes fitted means and standard errors for each condition (open and in vegetation). To test for a difference between these means we used *t*-tests according to Bailey (1976) to compare beetle numbers in trap logs between locations. All data analysis was performed using the statistical package R, version 2.10.0 for Windows (R Development Core Team.

2009. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria)

3.2.2   Effects of Non-host Volatiles on Attack by *Hylastes ater* of *Pinus radiata* Seedlings

Five sites were selected from a single area of second rotation *Pinus radiata* forest in Waipori Forest (managed by City Forests Ltd.) south of Mosgiel, Dunedin. This area was chosen as it had been felled between June to August 2008 and then re-planted during June to July of 2009 and because it was close to the adjacent Berwick Forest (managed by Wenita Forest Products Limited) where considerable *H. ater*-damage to first-year *P. radiata* seedlings was observed in 2008 (Figure 3.1). A detailed assessment of seedling damage could not be achieved in 2008 because no seedlings had been available for this purpose, which involves a destructive sampling method. City Forests Ltd. agreed to assist with this project in 2009, providing an area in Waipori Forest and the resources to plant 500 additional *P. radiata* seedlings for a subsequent assessment of attack by *H. ater*.
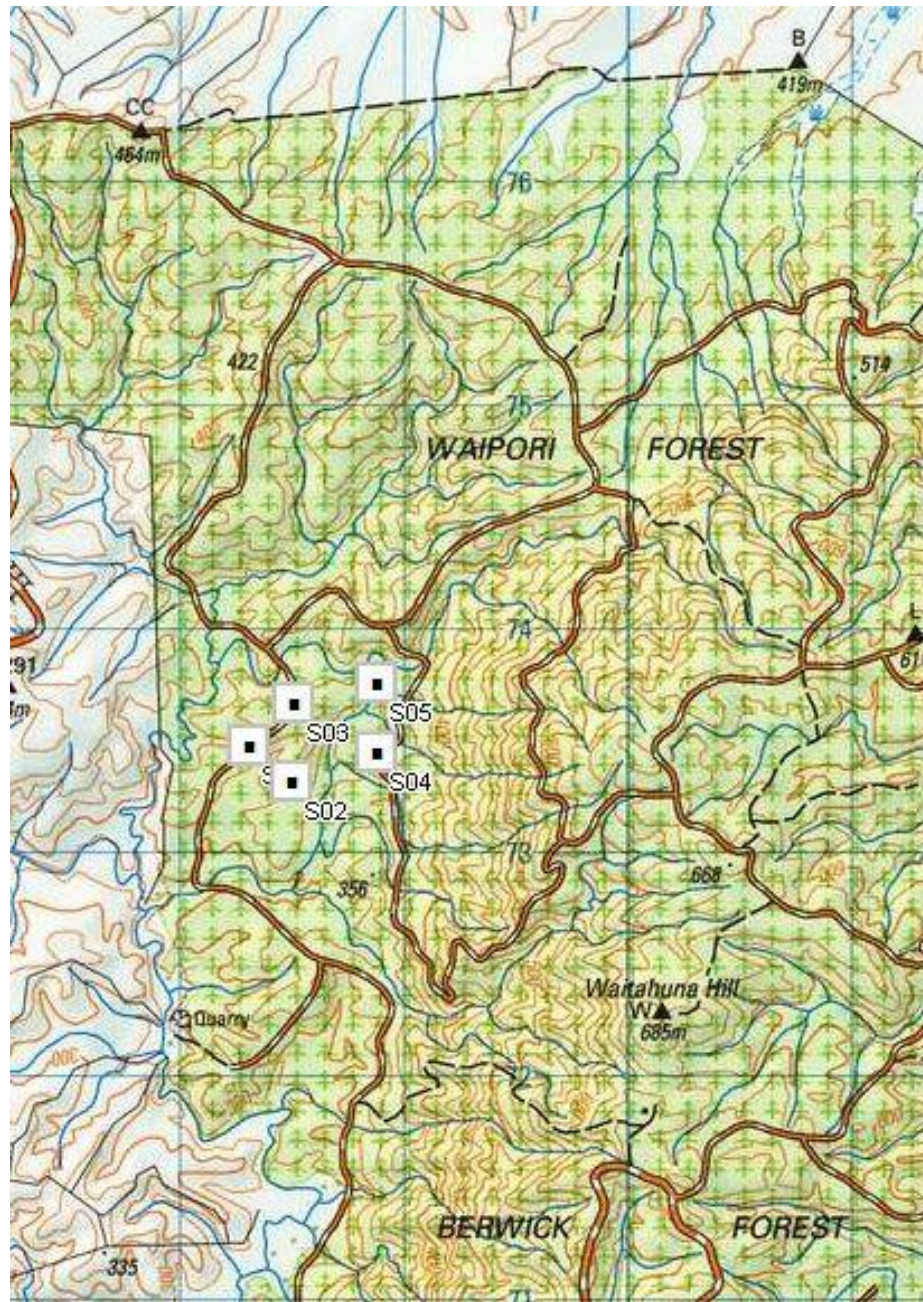
Figure 3.1 Site location (site 1; latitude -45.905; longitude 169.887; altitude 338 m) in Waipori Forest, which is approximately 50 km south of Dunedin, Otago, New Zealand. Map extracted from NZMS 1:50,000 series; blue grid lines are 1 km apart.

The seedlings were planted on 30 July 2009. This was before the beginning of the experimental manipulations (due to the availability of the City Forests Ltd. planters), but still before the start of *H. ater* flight activity. The trial was established in five replicate sites (Figure 3.1), with 100 seedlings planted 1 m apart in a single row at each site. Trial seedlings were planted between rows of previously planted existing seedlings. Two Lindgren flight intercept traps were installed at each site, as explained above and baited with attractant containing, 150 ml of ethanol and 150 ml of alpha-pinene. Traps were used to monitor *H. ater* flight activity during the trial, and they were cleared monthly over the trial period.

To assess the effects of non-host volatiles on attack by *H. ater* of *P. radiata* seedlings, three treatments were used; non-host green leaf volatiles (GLV), insecticide (for details see below), and blank control.

Insecticide treatment consisted of seedlings sprayed with Confidor® 5GR (Bayer AG, Germany), which contains Imidacloprid as active ingredient. Five gram sachets of Confidor® containing 5% Imidacloprid were mixed with 5 litres of water. Imidacloprid is a systemic insecticide which enters the plant through the cuticle, and it kills insects feeding on the treated plant. As systemic insecticides take time to fully penetrate a plant's system, another insecticide, Orthene® liquid (Monsanto Co., USA), was added at the first application. The active ingredients in Orthene® liquid are a combination of Acephate, at a concentration of 195 g per litre in the form of a soluble concentrate, and 346 g per litre of ethylene glycol. Orthene® liquid was added in the quantity of 5 ml to 5 l water into the Confidor® insecticide spray, as it works through direct contact to the insect. The combined insecticide was applied using a 5-litre hand pump sprayer unit. Insecticide was

applied to the base of each seedling, covering the soil, the stem and foliage according to the manufacturer's specifications, and reapplied three times at monthly intervals.

The GLV treatment consisted of seedlings treated with a GLV mixture described for the log experiment (see above). Approximately 5 ml of GLV mixture was applied per tree, containing 1.5 ml GLV, then reapplied three times at monthly intervals.

Each of the five replicate sites had 100 seedlings, and the treatments were applied from the start of planted rows as follows:

1. 33 seedlings treated with insecticide followed by;

2. 33 seedlings treated with green leaf volatile mixture followed by;

3. 34 seedlings blank (untreated) controls.

The first treatments were applied on 25 August 2009. The numbers of seedlings per treatment and sampling dates are shown in Table 3.1.

Table 3.1 The number of *P. radiata* seedlings established per treatment and the number removed at each sampling time during the trial, numbers per site are in parentheses. At the second date all remaining seedlings were removed.

Treatment	Total Established	Removed 25/09/2009	Removed 15/12/2009
Control	170 (34)	55 (11)	115 (23)
GLV	165 (33)	55 (11)	110 (22)
Insecticide	165 (33)	55 (11)	110 (22)
Grand Total Sampled	500 (100)	165 (33)	335 (67)

As the phyto-toxicity of the GLVs (E)-2-hexen-1-ol & (Z)-3-hexen-1-ol in mixture with silicon oil was unknown for *P. radiata* seedlings a nursery trial was setup at the start of August 2009 (before the field trial), to assess whether the GLV mixture or the silicon oil by itself damage seedlings.

The treatments in the nursery trial were as follows:

1. 10 seedlings sprayed with GLV mixture (70% oil to 30% GLV) covering the stem and foliage;

2. 10 sprayed with GLV mixture on the stem only;

3. 10 sprayed with silicon oil covering the stem and foliage'

4. 10 blank (untreated) controls.

Every week 5 ml of each treatment was applied, and any signs of damage on the plants noted. Two weeks after the start of the nursery trial, seedlings in treatment 1 (GLV applied to stem and foliage) started to show signs of damage, where the foliage had started to brown off. With this in mind the GLV mixture was applied only to the stem area and around the base of the seedlings in the field trial, while minimising the amount

applied to foliage. One month after the start of the nursery trial the seedlings in treatment 2 also showed similar signs of damage with the foliage browning off where the spray had touched. Therefore, from the second month of field application the GLV mixture was limited to the soil at the base of the trial seedlings.

Hylastes ater attacks by feeding on the root collar, roots and lower stem of host seedling trees (Clark, 1932; Crowhurst, 1969; Zondag, 1982; Ciesla, 1988; Reay, 2001; Reay & Walsh, 2002b). Due to the nature of this feeding behaviour, seedlings have to be removed from the ground in order to assess the extent of damage that has occurred and to confirm the likely cause of any above-ground symptoms. The severity of attack was graded as a percentage of maximum damage possible. This was done using the assessment guide to grade *H. ater* damage to roots, root collar and stem of *P. radiata* seedlings developed by Reay (2001). However, the grades used by Reay (2001) were revised to allow an estimate of the proportion of damage rather than assigning a grade, categories were as follows;

- 0 – No evidence of any attack,

- 10 – 30% – Low amount of damage, one or two small marks from feeding attempts. No sustained feeding.

- 40 – 100% – High damage, many small attempts to feed or long sustained feeding track, visible frass around the root collar, roots or stem area.

In order to grade the damage sustained during the peak flight of *H. ater*, seedlings were removed at two sampling times. The first, on 25 September 2009 (2 months after planting) one third of all seedlings were destructively sampled. The seedlings were bagged individually and taken back to the laboratory for assessment of damage. The

second and final sampling was on 15 and 16 December 2009 where all remaining seedlings were removed. Due to the larger number of seedlings (335) it took two people two days to remove all the seedlings for the return to the laboratory for assessment. The seedlings were stored in a freezer at -20° C before grading to ensure that no further insect damage occurred.

At both destructive sampling times a mortality assessment was conducted on all trial seedlings. Mortality was given as the percentage of browned-off foliage as follows:

- 0 – All foliage was green and apparently healthy,

- 50% - including all plants between 10 and 90% brown foliage,

- 100% of the foliage was completely brown, the seedling was apparently dead.

Data analysis

The effects of sampling time and treatment on the proportion of seedlings attacked by *H. ater* were investigated through a Generalized Linear Model with a binomial error distribution. Then, differences in the severity of attack between treatments were investigated with a Pearson's Chi-square test for independence, using only seedlings which had *H. ater* attack so that this analysis was independent of the one above. Due to low total numbers of seedlings with *H. ater* attack, the Chi-square test was confirmed through a Fisher's exact test for count data. All data analysis was performed using R version 2.10.0.

3.3   Results

3.3.1   Effects of Non-host Volatiles on Attack by *Hylastes ater* and *Hylurgus*

                 ligniperda of *Pinus radiata* Logs

Peak spring flight of *H. ater* and *H. ligniperda* in Chaneys Forest started in late
September through October 2009 (Figure 3.2). Numbers of *H. ater* declined towards the
end of the trial period in November, about the time when the trap logs were removed from
the field.
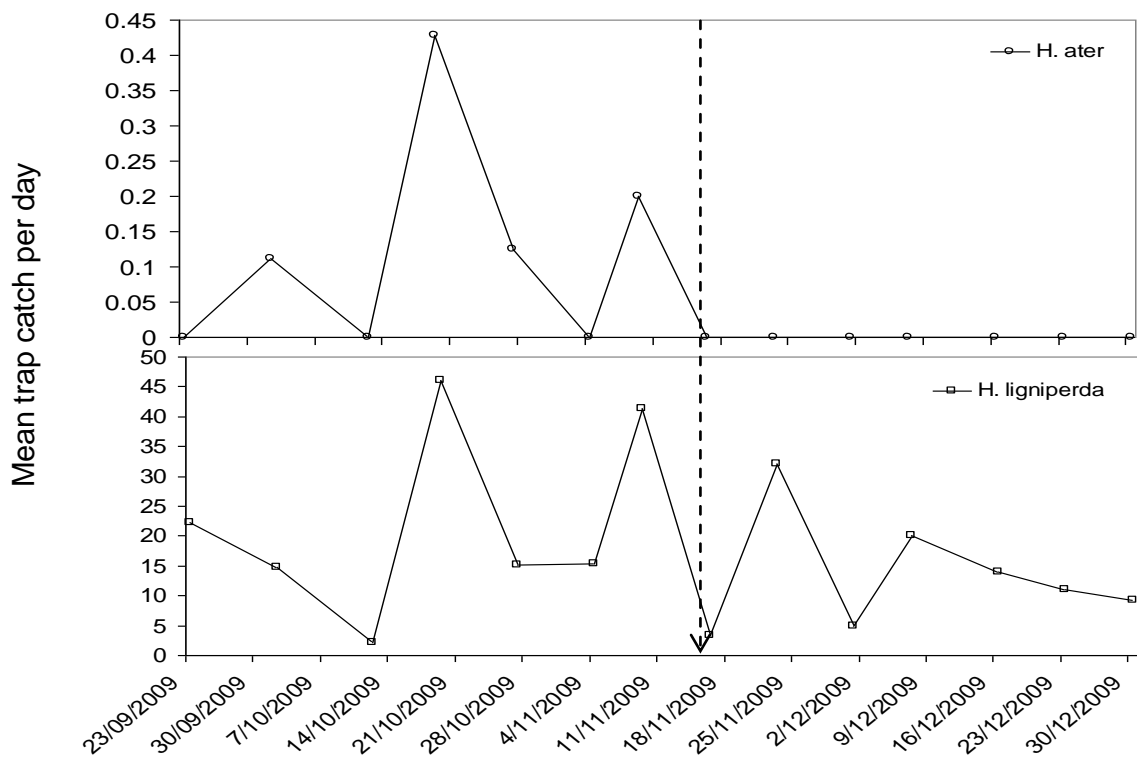
Figure 3.2 Flight intercept trap catch, mean trap catch per day of *Hylastes ater* and *Hylurgus ligniperda*
in Chaneys Forest from 23 September 2009 to 30 December 2009. Dashed line at 16 November 2009 is
the end of the trial when all logs were removed from the field.

Topically applied non-host volatiles

Logs treated with non-host volatiles, as a mixture of green leaf volatiles (GLV) in silicon oil, contained about half the number of *H. ater* found in control logs (Figure 3.3). However, according to a QuasiPoisson generalised linear model, the difference was not or only marginally significant (Table 3.2). For *H. ligniperda*, the GLV treatment had a similar, but significant effect (Table 3.2, Figure 3.3).

Table 3.2 Results from two Quasipoisson Generalized Linear Models testing the effects of treatment (GLV treated logs versus untreated logs) on the number of adult *H. ater* or *H. ligniperda* on the trap logs..

		Df	Deviance	Resid. Df	Resid.Dev	F	Pr(>F)
Hylastes ater	NULL			49	105.2		
	treatment	1	8.7	48	96.5	3.49	0.07
Hylurgus ligniperda	NULL			49	404.1		
	treatment	1	45.0	48	359.1	5.55	**0.02**

*P-values in bold indicate significantly different means
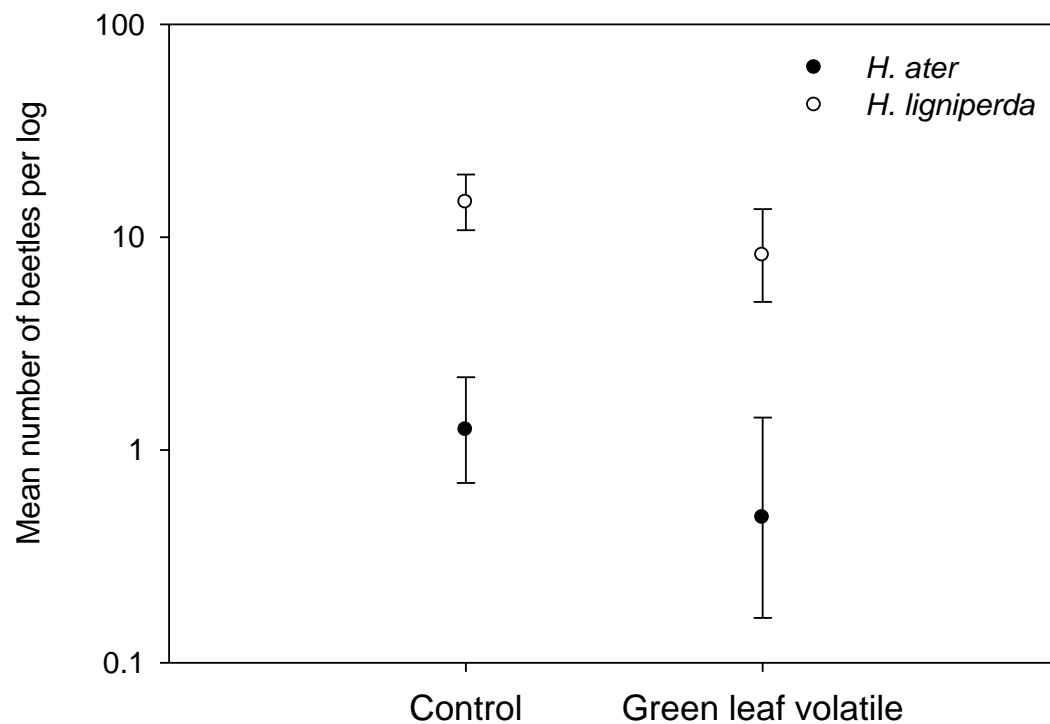
Figure 3.3 Fitted mean number (+/- 95% CI) of *H. ater* and *H. ligniperda* found on trap logs with different treatments; untreated (control) logs; and Green leaf volatile treated logs. Y-axis plotted on a log scale.

Naturally emitted non-host volatiles

The comparison of bark beetle attacks of trap logs in open areas and in areas with an understorey of broadleaved shrubs (which naturally emits non-host volatiles) gave mixed results. For *H. ater* t-tests on the GLMM means found there was no significant treatment effect of logs being among broadleaved vegetation compared with logs in the open (Table 3.4, Figure 3.4). However, placing trap logs among broadleaved vegetation significantly reduced *H. ligniperda* numbers, by about 75% compared with logs in the open forest (Table. 3.4, Figure 3.4).

Table 3.3 Results from Quasipoisson Generalized Linear Mixed Models and subsequent *t*-tests testing the effects of treatment, logs out in the 'Open' forest or within non-host broadleaved 'Vegetation' on the number of adult *H. ater* or *H. ligniperda* per trap log.

	Treatment	Fitted Mean	Std. Error	t - value	*P*
Hylastes ater	Open	0.13	0.40	1.02	NS
	Vegetation	0.22	0.07		
Hylurgus ligniperda	Open	1.50	0.52	9.72	**< 0.05**
	Vegetation	0.35	0.16		

*t-values denoted in bold indicate significantly different means
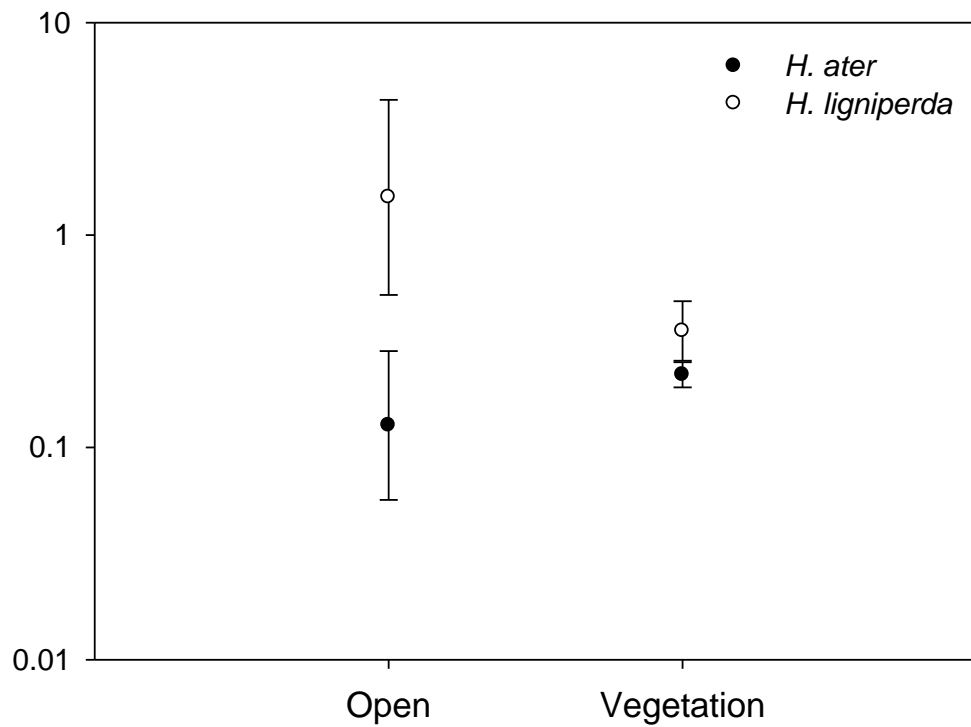
Figure 3.4 Fitted mean number (+/- 95% CI) of *H. ater* and *H. ligniperda* found to attack trap logs within different treatments; logs in 'Open' areas of the forest and logs within non-host broadleaved 'Vegetation' that natural emits non-host volatiles. Y-axis plotted on a log scale.

3.3.2   Effects of Non-host Volatiles on Attack by *Hylastes ater* of *Pinus radiata*

Seedlings

Activity of Hylastes ater during seedling trials

Field trials to assess attack of *P. radiata* seedlings by *H. ater* (Figure 3.5) were undertaken in Waipori forest, south of Dunedin, Otago. Over the period of this trial, peak flight of *H. ater* occurred in September (Figure 3.6), and flight activity declined towards the end of the trial period in December 2009.

Figure 3.5 *Hylastes ater* feeding on the stem of a *Pinus radiata* seedling in Berwick forest, Dunedin (2008).
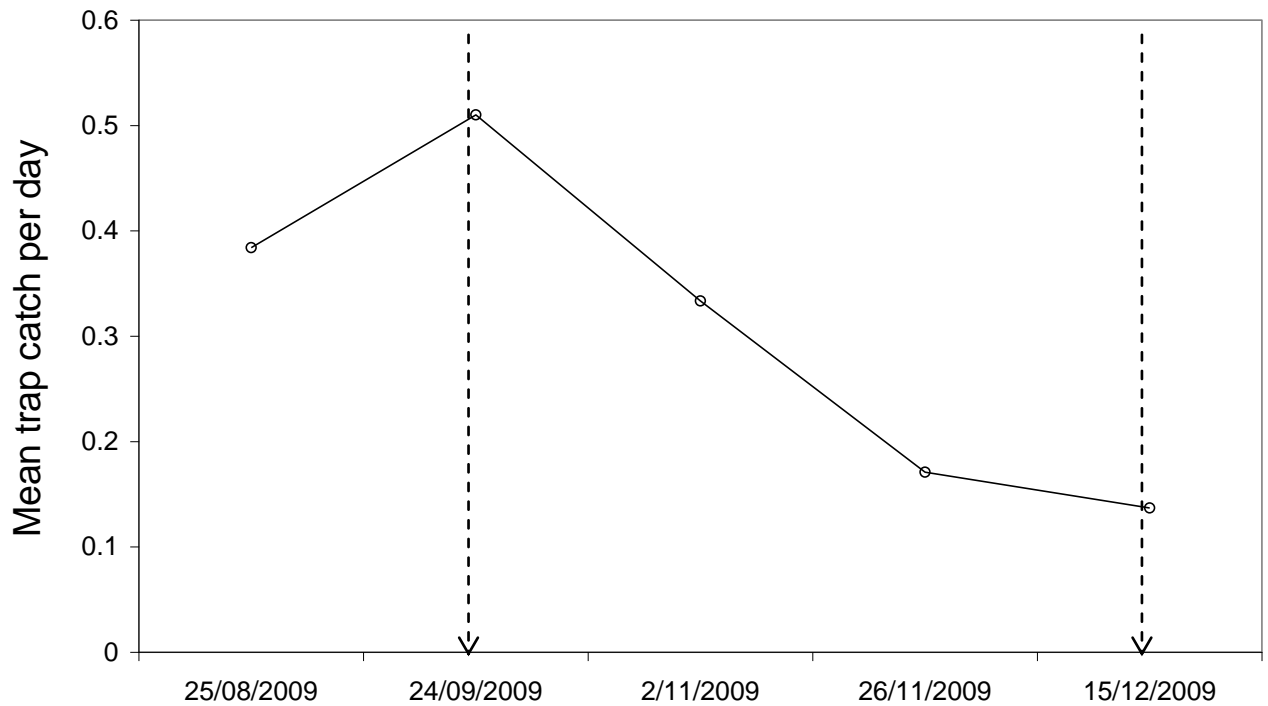
Figure 3.6 Mean number of *Hylastes ater* individuals caught per trap per day in flight intercept traps during the trial period from 25 August to 15 December 2009 in Waipori Forest, Dunedin. The dashed lines represent 25 September and 15 December 2009 when seedlings were sampled.

Seedling mortality and attack

The first measure of bark beetle attack of seedlings was field assessments of seedling mortality, which showed a large difference among treatments (Table 3.4). This was mostly driven by the fact that the green leaf volatiles (GLV) appear to have a phyto-toxic effect when directly applied to the seedlings, with 75 out of 165 treated seedlings classed as dead (Table 3.4). This was matched by the results from the nursery trial where all twenty GLV treated seedlings died. By contrast, the control and insecticide treated seedlings sustained low levels of mortality during the trial. Therefore, the mortality data appeared to be an artefact of treatment, rather than related to beetle attack.

Table 3.4 Total numbers of seedlings in different seedling mortality and foliage browning categories over both sampling times (25 September and 15 December 2009).

Treatment	0 (Green)	50 (Browning off)	100 (Completely brown)	Grand Total
Control	167	1	2	170
GLV	55	35	75	165
Insecticide	157	5	3	165
Grand Total	379	41	80	500

The second variable that was assessed was the proportion of seedlings attacked. Only a small number of seedlings showed signs of attack by *H. ater* of the roots, root collar or stem (23 out of 500) (Table 3.5), and GLV-treated seedlings were the most attacked, while those in the control treatment showed the least attack (Table 3.5). According to a Binomial generalised linear model, treatment (insecticide, GLV, and control) had a significant effect on the observed proportion of *H. ater* attack (Table 3.6). The time of sampling (i.e., removal of seedlings) and the interaction between sampling time and treatment were not significant (Table 3.6). The significant treatment effect was mostly driven by the GLV treatment, with 13 out of 165 GLV-treated seedlings attacked (and 13 out of 23 seedlings that were attacked across all treatments) (Figure 3.7). It is not known whether the higher beetle attack was because of attraction to the GLVs, or attraction to dying seedlings caused by GLV damage (see above).

Table 3.5 Total number of *Pinus radiata* seedlings attacked by *H. ater* over two sampling times with different treatments.

Treatment	25/09/2009	15/12/2009	Grand Total
Control	1/55	3/115	4/170
GLV	3/55	10/110	13/165
Insecticide	2/55	4/110	6/165
Grand Total	6/165	17/335	23/500

Table 3.6 Results from Binomial Generalised Linear Model testing the proportion of *H. ater* attack on *Pinus radiata* seedling with treatment (Control, Green Leaf Volatiles, and Insecticide) over two sampling times, 25/09/2009 and 15/12/2009.

	Df	Deviance	Resid.Df	Resid.Dev	P(>|Chi|)
NULL			499	186.56	
Sampling Time	1	0.54	498	186.02	0.46
Treatment	2	6.13	496	179.89	**0.047**
Sampling Time:Treatment	2	0.24	494	179.65	0.89

*P-values in bold indicate significantly different means
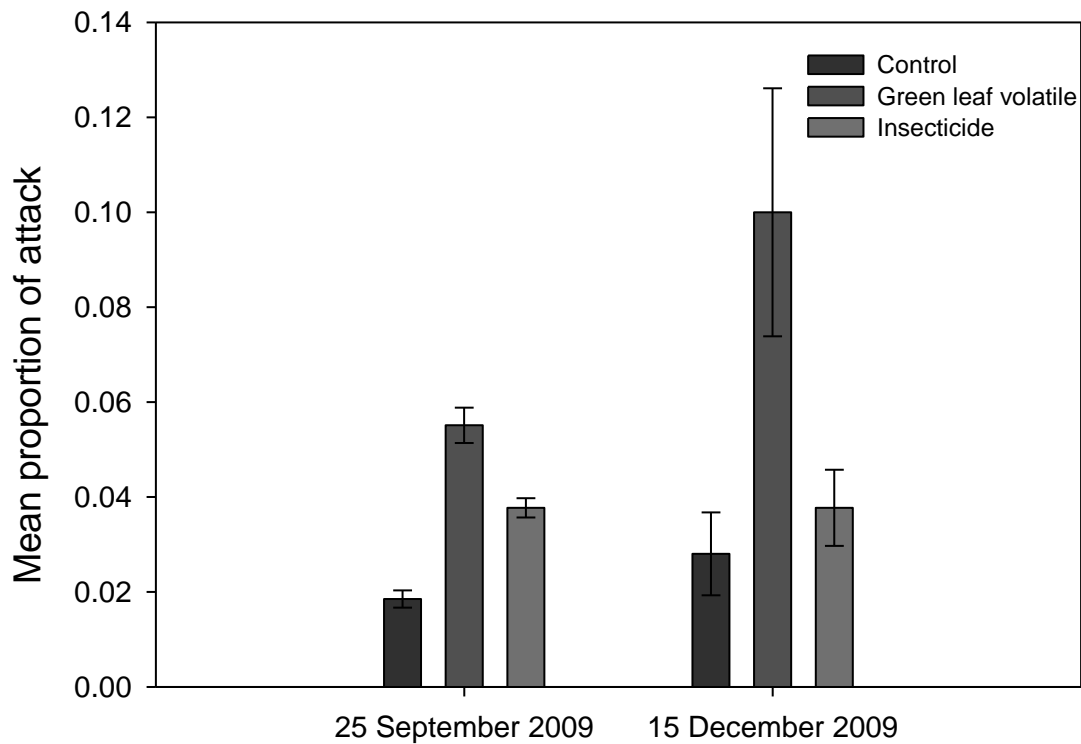
Figure 3.7 Fitted mean number (+/- 95% CI) from Binomial generalised linear model testing the proportion of *H. ater* attack on *Pinus radiata* seedling with treatment (Control, GLV, and Insecticide) over two sampling times, 25/09/2009 and 15/12/2009.

Severity of Attack

The third variable that was assessed was the severity of attack given that *H. ater* was present on *P. raditata* seedlings. Here, treatment had a significant effect on the severity of damage when assessed with a Chi-square test ($X^2 = 10.75_{(2)}$, P=0.004). Due to the low numbers in some cells, a Fisher's Exact test for count data was performed, and this confirmed a significant treatment effect ($P = 0.001$). Again, this difference appeared to be driven by the prevalence of attack and high amounts of damage found on the GLV-treated seedlings (Table 3.7).

Table 3.7 The total number of seedlings attacked with category of damage caused by *H. ater*. 'Low damage' to seedlings is the combined count for 10 - 30% damage, and 'High damage' to seedlings is the combined count for 40 - 100% damage to areas of seedlings (roots, root collar, and stem).

Treatment	Low damage	High damage	Total
Control	3	1	4
GLV	3	10	13
Insecticide	6	0	6
Total	12	11	23

3.4   Discussion

3.4.1   Effects of Non-host Volatiles on Attack by *Hylastes ater* and *Hylurgus ligniperda* of *Pinus radiata* Logs

Topically applied green leaf volatiles (GLV) (2-Hexen-1-ol, 3-Hexen-1-ol in carrier silicon oil) were found to have a repellent effect on bark beetle attacks. These non-host volatiles significantly reduced attacks of *P. radiata* trap logs by *H. ligniperda* and also appeared to reduce attacks by *H. ater*, though the latter effect was marginally non-significant, possibly because of the comparatively small number of *H. ater* that was available for this analysis. The results suggest at least partial protection of logs from attack by *H. ater* and *H. ligniperda* is possible by applying green leaf volatiles. However, this did not stop attack completely, and such a partial, rather than absolute, treatment effect has also been demonstrated for ambrosia beetles (Borden, et al., 2001), and mountain pine beetle (Huber & Borden, 2001b). A stronger effect was observed in a laboratory study of the response of *A. ferus* to the same green leaf volatiles applied to host

burnt logs (Suckling, et al., 2001). The greater rate of evaporation in the field of GLVs may have reduced the treatment effect over time. More frequent application could have been needed over the eight and a half weeks of the trial. Alternatively, the disruption by GLV from dispensers applied at a higher density may achieve a longer lasting and stronger effect, as was proven through both log and trapping research with other wood-boring and bark beetle species; the mountain pine beetle (*Dendroctonus ponderosae*), the European spruce bark beetle (*Ips typographus*), the ambrosia beetles, (*Gnathotrichus sulcatus* and *Trypodendron lineatum*) and the burnt pine long horned beetle (*Arhopalus ferus*) (Delglow & Borden, 1998; Borden, et al., 2001; Huber & Borden, 2001a; Suckling, et al., 2001; Q. H. Zhang & Schlyter, 2003). The difference in abundance of *H. ater* and *H. ligniperda* highlights their population levels with in the forest. *Hylurgus ligniperda* has proven to be the dominant species around New Zealand (Chapter 2; Reay, 2001). *Hylurgus ligniperda* has shown to be strongly influenced by olfactory cues, through disruption by the application of green leaf volatiles to *P. radiata* logs and dispensers on host mimicking monitoring traps in the previous trapping trial (Chapter 2).

In the trial assessing effects of naturally emitted non-host volatiles on attack of bark beetles, the results indicate that logs among broadleaved vegetation within *P. radiata* forests were attacked less than logs in open areas, in the case of *H. ligniperda*. There was no significant change in number of *H. ater* among broadleaved plants, but the small sample size of this species limits the power to detect any potential differences. Observations from the flight intercept traps which caught a total of just six *H. ater* over the eight and a half weeks of the trial period confirmed that this species was comparatively rare. Furthermore, an unbalanced trial design caused by the disappearance of 6 trap logs of which the majority were taken from one site that had a higher infestation

rate, probably reduced the power of the experiment even further. The results show that non-host volatiles applied topically or emitted by non-host broadleaved plants within conifer forests can reduce the prevalence of attack by *H. ligniperda* and probably also *H. ater* of *P. radiata* logs. This effect warrants further investigation towards practical application in pest management practices.

3.4.2   Effects of Non-host Volatiles on Attack by *Hylastes ater* of *Pinus radiata* Seedlings

The results of the trial comparing the proportion of attack by *H. ater* of *P. radiata* seedling were unexpected. Although there were significant differences between seedlings treated with insecticide or green leaf volatiles, and untreated controls, the 'unprotected' seedlings were the healthiest. However, only a small number of trial seedlings showed signs of attack to the areas of the roots, root collar and stem. Only 23 out of 500 trial seedlings (4%) were attacked by *H. ater,* which is low level of attack compared to reports at other sites of up to 50% attack (Reay, 2001; Reay & Walsh, 2002b). My observations on seedling attack rates correspond well with the low catches of *H. ater* in the flight intercept traps, suggesting that in the year of this trial, *H. ater* populations were low in Waipori forest. This limits the ability to draw conclusions about the effectiveness of these treatments with regards to attack by *H. ater*. Nevertheless, *H. ater* attack of seedlings treated with the insecticide (Confidor®) or GLV (2-Hexen-1-ol, 3-Hexen-1-ol in silicon oil) suggests these treatments did not provide an effective means of protection. My finding that the control group suffered the least attack by *H. ater* contrasts with previous

research (Reay, 2001; Reay & Walsh, 2002b). However, these findings are partly confounded by other factors which mask the effects of these treatments on 'healthy' seedlings (see below).

Sampling time for the removal of seedlings and the interaction between sampling time and treatment had no significant effect on the proportion of seedlings attacked by *H. ater*. The significant treatment effect was largely driven by the high proportion of attack of the GLV-treated seedlings, with 13 out of the total of 23 attacked seedlings. These results had been swayed by the effects of the GLV treatment which clearly damaged and killed seedlings in both the field and the nursery trial. The decline of seedling health was visible during the trial as the foliage of the seedlings turned brown from the apparent phyto-toxicity. *Hylastes ater* is known to preferentially attack stressed or weakened seedlings (Zondag, 1968, 1982), and the results supports these findings. The main effect was that the GLV treatment weakened or killed the *P. radiata* seedlings. It is therefore highly likely that the trial seedlings were attacked post application with repellent GLV. Potentially, if there was any repellent effect it could have worn off between application times allowing *H. ater* to attack the already susceptible seedlings.

It was anticipated that treatment with insecticide would have protected the seedlings from attack as has been reported in previous research with *H. ater* and other *Hylastes* species (Dowding, 1973; Ciesla, 1988; Lindelöw, 1992; Leather, et al., 1999; Allan & Higgs, 2000; Allan, et al., 2000; Reay & Walsh, 2002a; Rolando & Allan, 2004; Rolando, 2006). However, the insecticide treated *P. radiata* seedlings sustained attack from *H. ater*, but compared to GLV the amount of damage from an attack was low. It is possible that the limited damage of some seedlings would not have led to their death, and that they would

have survived if this had occurred under normal circumstances in the forest environment (Reay, 2001).

Further information and research is needed to fully understand the implications of using green leaf volatiles topically as repellents on *P. radiata* due to the apparent phyto-toxic nature of 2-hexen-1-ol and 3-hexen-1-ol leaf alcohols in oil solution that was witnessed during the trial. Potential management solutions could come from the use of chemical dispensers with green leaf volatiles (or other repellents) to protect the seedlings from direct contact with chemicals, a method that has proven effective for logs (Borden, et al., 2001; Huber & Borden, 2001b). Another option could be to use a systemic insecticide that would also mask the host-volatiles which attract the beetles (Leather et al., 1999). The effects of natural sources of non-host volatiles from broadleaved plants within the forest are promising. These would mask both the host-volatiles and the visual cues from the shape of seedlings. Natural non-host volatiles have shown potential to disrupt bark beetles attacking trap logs within broadleaved vegetation in the trap log trial. A review of the effects of forest biodiversity on insect pests has shown that mixed forests suffer less damage, overall, than single-species forests (Jactel and Brockerhoff, 2007). This could be a useful strategy to reduce the effects of bark beetles in New Zealand forests, while at the same time increasing forest biodiversity and, potentially, the conservation value of production forests. However, the value of broadleaved plants in reducing bark beetle attack would have to be balanced against their possible competitive suppression of the growth of pine seedlings. Further investigations are necessary to determine whether mixed forest strategies can be implemented.

4   GENERAL DISCUSSION

4.1   Introduction

Olfactory and visual cues used for host finding by the bark beetles *H. ater* and *H. ligniperda* and the longhorn beetle *A. ferus* in *P. radiata* forests in New Zealand were evaluated in this study. These species of wood boring and bark beetles are among the most significant insect pests of plantation forestry in New Zealand, where they occupy somewhat similar ecological niches within the forest environment. The management of these pests incurs significant expenditure to reduce seedling attack and to ensure timber and logs for export are free of these pests.

Hylastes ater, H. ligniperda and *A. ferus* responded positively to the presence of host volatiles on monitoring traps (Chapter 2). The study of olfactory and visual cues in host finding show that host specific cues, with attractant host volatiles and host-characteristic, silhouette or dark colour are attractive. Similarly, non-host cues including the green leaf volatiles (E)-2-hexen-1-ol and (Z)-3-hexen-1-ol and white silhouette are not, or less, attractive, although the relative effect of olfactory and visual non-host cues differed between species. *Hylastes ater* trap catch to host-characteristic cues (black traps with attractant) was over 10 times the catch to non-host cues (white traps with repellent). The trap difference between host and non-host cues was also present for *H. ligniperda*, though the difference between host-characteristic traps was over 100 times that of non-host. The response of *H. ligniperda* to traps with repellent was similar to *H. ater*, however there was a much larger response by *H. ligniperda* to host specific traps. There was no significant

repellent effect of GLV for *A. ferus* which was an unexpected result. A previous study of *A. ferus* found significant effects of GLV (Suckling, et al., 2001), however, that study used walking and ovipositon bioassays that tested close-range responses, whereas my trapping study assess responses of flying beetles. The significant effects of trap colour and relatively greater F-values, compared to attractant effects, as well as the greatly reduced catch in clear traps, suggest that visual cues are more important during host finding for *A. ferus* than in the two bark beetle species.

While *H. ater* and *H. ligniperda* occupy similar ecological niches, *H. ater* is known to cause substantial damage in *P. radiata* regeneration in New Zealand (Zondag, 1982; Reay, 2001; Reay & Walsh, 2002b). However, *H. ligniperda* is not known to attack seedlings (Bain, 1977), in New Zealand or in South Africa (Tribe, 1991) where species of *Hylastes* also cause damage. In Chile both species, *H. ater* and *H. ligniperda*, are present and are reported to cause damage to seedlings (Ciesla, 1988). The report of seedling attack by *H. ligniperda* in Chile could be a misinterpretation or misdiagnosis seedling death and damage when this was actually caused by another bark beetle species that occupies the same habitat. Such cases have been reported previously (Lindelöw, 1992, 1992a.; Orlander & Nilsson, 1999; Reay, 2001; Reay & Walsh, 2002b). Forestry reports in New Zealand suggest high levels of damage caused by *H. ater* (Clark, 1932; Zondag, 1968), however, results from the current study (Chapter 3) on seedling mortality in Waipori Forest, near Dunedin, where *H. ligniperda* are not, or not yet, present in large numbers suggest low levels of damage of about 4%. This supports previous research in New Zealand which reported relatively low levels of damage (5%) over larger sampling areas. Further research should aim at assessing seedling damage, to verify if high levels of

damage still occur, and if so to better characterise sites that are prone to more serious bark beetle damage.

The current study on *P. radiata* seedling attack was conducted in a region of New Zealand (Otago) that had very low numbers of *H. ligniperda* at the time (Chapter 3). *Hylurgus ligniperda* has only recently arrived there as its distribution is still expanding southwards since its arrival near Auckland. *Hylastes ater* is thought to be more problematic in regions with no or little competition from *H. ligniperda*, which occupies the same niche, than in areas where both species coexist (Reay, 2001). However, despite the comparatively greater abundance of *H. ater*, relative to *H. ligniperda*, this was not reflected in the amount of seedling damage in the year of my main seedling trial. During a preliminary assessment of seedling damage in the adjacent Berwick Forest during the previous year, 14 out of 20 seedlings were attacked by *H. ater*. Although this was based on a very small sample, which cannot be considered indicative of stand-wide attack, it suggests that *H. ater* can sometimes be problematic. The apparent competition between *H. ater* and *H. ligniperda* may decrease populations and therefore the risk from *H. ater* in the future. To my knowledge, the existence of this competitive relationship between these two species has not yet been demonstrated with certainty, and the exact nature of this remains to be investigated. However, in Europe, where *Hylastes ater* is also regarded as a pest of pine seedlings, the impact of this pest has apparently lessened due to the presence of a more aggressive species, the pine weevil *Hylobius abietis*, that damages seedlings and displaces *H. ater* (Lindelöw, 1992).

The current study assessed the practical application of the repellent qualities of green leaf volatiles typical of broadleaved plants, (E)-2-hexen-1-ol and (Z)-3-hexen-1-ol, to

influence attack of the conifer bark beetles *H. ater* and *H. ligniperda* when applied to cut logs and seedlings of *P. radiata*. While the use of green leaf volatiles did not stop all attack, it did decrease the attack of logs by 40-60% by both species. These results support findings from studies of other bark beetles which found volatiles from non-host plants (relative to the target insect) can reduce attack to logs and standing trees that were susceptible to bark beetle attack (Borden, et al., 2001; Huber & Borden, 2001b). Further research on the use of topically applied green leaf volatiles should assess the application process, potentially utilising chemical dispensers, which were used in the trapping trial in this project, to limit the volatilisation from direct application. This may lead to the development of a repellent for application in high risk forestry areas, for example, for the protection of logs before they are removed from harvested areas or at log storage areas at ports and mills.

Though the spraying of green leaf volatiles on seedlings had some unexpected phyto-toxic side effects which prevented assessment of the effect of non-host volatiles on beetle damage, the study allowed for the assessment of the severity of damage by *H. ater* on control (unsprayed) seedlings. Historically, studies in New Zealand have focused on seedling mortality rate rather than the extent of the damage that *H. ater* can cause (Zondag, 1968, 1982). My results supported previous research from the North Island of New Zealand that assessed the severity of *H. ater* damage as I found several cases of damage that would be classed as sub-lethal attack (Reay, 2001). However, as my sampling method was destructive, it is not possible to determine how much damage these seedlings would have sustained eventually. Nevertheless, this highlights that *H. ater* attack as such does not necessarily cause seedlings to die, and that it is also important to recognise the existence of other causes of mortality such as poor planting practice and

draught. As seedlings that have died from other causes can subsequently be attacked by *H. ater*, it is possible to misinterpret the presence of beetles on dead seedlings as the causal factor of seedling mortality. For this reason the insecticide treatment was added to my seedling trial, to enable a comparison of seedling mortality between unprotected 'control' seedlings and insecticide-treated seedlings, which were expected to be not, or less, attacked by *H. ater*. However, as the level of attack of 'control' seedlings was so low during the trial, it is not possible to draw any further conclusions about the risk of attack and the effectiveness of any treatments from my work.

In natural forest environments access to host material for secondary bark beetles, like *H. ater, H. ligniperda* and *A. ferus* is likely to be limited to occasional events, for example single tree fall, storm-broken branches or larger scale events such as windthrow, fire and outbreaks of primary bark beetle species which can kill vast quantities of trees leaving material for secondary bark beetles to colonise (Kirkendall, 1983; Raffa & Berryman, 1983; Raffa, et al., 1993). Secondary bark beetles are very successful at exploiting pulses of resources in the event of disturbance, which can result in populations reaching epidemic proportions, until they exhaust the available host material (Rudinsky, 1962; Christiansen, et al., 1987). Resources in natural forests are often limited and widely dispersed, but such limitations affect bark beetles that occupy plantation forests less, as resources are often plentiful from year-round harvesting. As a way of managing populations of insects that are prone to epidemics in plantation forestry, managers could consider the properties of natural mixed forest environments, which tend to suffer less from such pest problems (Jactel & Brockerhoff, 2007). The current study has shown that non-host plants that are present within *P. radiata* forests such as broadleaved 'weeds' can reduce the numbers of *H. ligniperda* that attack *P. radiata* logs. Further research on bark

beetle management needs to consider the natural mixed forest environment and the potential use of non-host species with properties that can contribute to the 'natural control' of bark beetle and wood borer populations that cause problems in plantation forestry. The species of non-host plant utilised in this project (primarily Scotch broom, gorse, and tree lupin) are classed as 'weeds' because they cause establishment problems and growth losses due to their competition with pines for light, nutrients and water. Also, these introduced exotic plants can have other detrimental effects in forest environments (Atkinson & Cameron, 1993). These plants were utilised in my project due to their frequent occurrence and abundance in plantation forests. Ideally, the information gathered during this project would be transferred to the use of other non-host plants that can produce a disruptive effect without affecting the growth of plantation trees in a mixed forest environment. There is potential to integrate economically productive species that could offer the visual and olfactory protection that has been found with 'weed' species.

4.2   Control Options for *H. ater, H. ligniperda* and *A. ferus,* and the Direction of

Future Research.

This thesis addressed the relative importance of the mechanisms and cues that are involved in host finding of *Hylastes ater*, *Hylurgus ligniperda* and *Arhopalus ferus* and how these can be disrupted by presenting inappropriate (non-host) cues to these insects. The use of visual and olfactory host and non-host cues could provide greener tools for managing these pests, by reducing or preventing attack of seedlings and timber.

The intra-specific interactions between *H. ater, H. ligniperda* and *A. ferus* are important for plantation forestry management to consider as these species all compete for similar breeding substrate within *P. radiata* forest. The interaction between these species are an important aspect for further research, as early observations from (Clark 1932), and Crowhurst (1969) show that prior to the introduction of *H. ligniperda* there were potentially higher numbers of *H. ater* present in New Zealand forests than found in recent research, which now show a clear dominance of *H. ligniperda* during the spring and summer adult flight season (Chapter 2; Reay, 2001).

Options for control for *H. ater, H. ligniperda* and *A. ferus* should focus on the biological and environmental factors that affect their population size, for accurate identification of high-risk areas, during the high risk times of the year, between February and May when the adult beetles are in full flight seeking new host material. The differences reported (Chapter 2) of seasonal flight activity of *H. ater, H. ligniperda* and *A. ferus* mean it is possible to make predictions of high risk periods during the year, enabling forest

managers to structure planting and harvesting events accordingly to lessen the risk of attack to vital resources.

Methods of protection against pest species of bark beetle can include various measures to reduce pest insect abundance in plantation forest environments (Lanfranco, et al., 2004). These can include, for example, host plant destruction, the removal of known host material, including the rapid removal of harvest logs from clear-fell areas, the application of pesticides, and the use of biological control agents. During log storage, logs have been found to be less susceptible to bark beetle attack if they are elevated off the ground and stored within the plantation, underneath standing trees rather than on clear-fell or skid sites which attract wood boring and bark beetles (Mausel, et al., 2007). The incorporation of trapping methods has increased in use in more recent years especially for more aggressive wood borers and bark beetles, using techniques such as mass trapping with trap logs. Cut logs of host trees attract pest species which are allowed to colonise the logs which are then destroyed, debarked, or treated with insecticides, to control the insects. In the case of aggressive primary species, standing trap trees can be used for the same purpose (Huber & Borden, 2001b; Shelton & Badenes-Perez, 2006). The use of trap logs and of traps such as those used in my project, for monitoring the abundance of bark beetles with in forests is well established (Clark, 1932; Tribe, 1991; Borden, et al., 2001; Huber & Borden, 2001a; Suckling, et al., 2001; Mausel, et al., 2007). The use of mass trapping for area-wide control or for eradication programmes suffers from some conceptual problems. For example, trap saturation can be a problem when populations are large. Consequently, mass trapping has rarely been able to solve a pest problem, and it is unlikely to be successful as a sole method of control for large expanses of plantation forests as in New Zealand (Brockerhoff, et al., 2010b). Control techniques available for

protection against bark beetles, are forestry focused and more specifically aimed on the protection of seedling trees (Allan & Higgs, 2000; Reay & Walsh, 2002a; Rolando, 2006). Forest management, through the removal of host material and planting of seedlings during times when the risk from attack is lower, potentially combined with the use of insecticides pre- and post- planting minimize *H. ater, H. ligniperda* and *A. ferus* damage within forestry at the present. One option that is commonly practiced in New Zealand involves delays in planting after harvest of one to two years, to protect seedlings from beetles that breed in harvesting slash (Reay, et al., 2005; Reay, et al., 2008). However, this delay comes at a high economic cost, increasing length of time until the following harvest.

International phytosanitary regulatory standards for the quarantine of export of logs, timber and wood products have been implemented to limit the introduction and export risk of known wood boring or bark beetles species (Brockerhoff, et al., 2006a). Management of wood boring and bark beetles within export timber aims to develop more environmentally friendly options to the fumigants and pesticides that are currently used for pest control (Reay & Walsh, 2002a; Z. Zhang, et al., 2004). To reduce the need for toxic environmentally harmful chemicals, recent research to reduce the presence of wood borers and bark beetles in high risk areas has investigated the use of 'push-pull' lighting strategies, utilising attractive ultraviolet 'pull' light traps and unattractive yellow (high and low pressure sodium) and white (metal halide) 'push' lighting (Pawson & Watt, 2009). This research showed that specific light spectra (yellow light) combined with UV light traps placed next to existing lights can have a significant effect on the number of beetles. In developing this method into a future management strategy further research is needed in larger scale trials that also consider the incorporation of other stimuli that

influence beetles in high risk areas, such as olfactory stimuli from semio-chemicals that are present at wood processing areas (Suckling, et al., 2001; Pawson & Watt, 2009), to be able to assess the potential effect of using modified lighting to influence populations of wood borers and bark beetles.

There is a positive relationship between the attack of logs and seedling trees by wood borers and bark beetles and the occurrence of sapstain fungi after attack (Dowding, 1973; Klepzig, et al., 1996; Paine, et al., 1997; Suckling, et al., 1999; Reay, et al., 2005). There is currently on-going research in the interaction between wood-boring and bark beetle species and the presence of sapstain fungi which has many implications for New Zealand's forest industry (pers. comm.). Further research is required to properly understand the interactions between attack by bark beetle species and the invasion of timber and seedling trees by fungi, as it has been shown that fungi can be present in seedlings after attack by *H. ater*, where 50-80% of severely damaged seedlings have been found to carry sapstain fungi (Reay, et al., 2002; Reay, et al., 2005). Therefore, the dynamics of fungal invasion and the long-term effect this can have on subsequent tree growth need to be properly understood.

Opportunities exist for forest management to incorporate host and non-host plant specific cues into management of wood-boring and bark beetle species. For example, further research into seedling plants that prove to be less susceptible to *H. ater* attack (Reay, 2001) and the use of non-host plants in the protection of newly planted seedlings or logs. Host-specific attractants and repellents have been identified as effective in monitoring programmes and need more research to explore their potential use for large-scale control. The present study found a reduction in the attack of both *H. ater* and *H. ligniperda* on *P.*

radiata logs when non-host green leaf volatiles were topically applied (Chapter 2), supporting research with non-host volatiles which found the protection of wood and trees from other bark beetle species (Borden, et al., 2001; Huber & Borden, 2001b). Such research shows promise for practical application of green leaf volatiles or other non-host volatiles from dispensers or naturally occurring broadleaved plants within mixed forest environments. Incorporating both visual and olfactory barriers in disrupting host finding of bark beetles to reduce attack on cut logs or seedling plants in high risk areas like clear fell, log storage in the forest or within mills and port situations could help to reduce damage done by wood-boring and bark beetle species.

Mixed forests increase the density of different tree species presenting a more complex environment to foraging insects. In a mixed forest, suitable host trees are more widely spaced and represent a smaller proportion of trees which results in physical as well as chemical barriers from non-host volatiles, both of which can interfere with host location. Disruption of host location has been shown to occur with the application of non-host plant cues on the conifer feeding bark beetles, *H. ater* and *H. ligniperda* in this project (Chapter 2 & 3) and *A. ferus* (D. M. Suckling et al. 2001), and other bark beetle species (Byers, et al., 1998; Borden, et al., 2001; Huber & Borden, 2001b, 2001a; Strom, et al., 2001; Byers, et al., 2004; Goyer, et al., 2004; Q. H. Zhang & Schlyter, 2004; Campbell & Borden, 2006b, 2009). Jactel and Brockerhoff's (2007) analysis of mixed forest effects has shown that different species of trees can produce visual and olfactory barriers of non-host cues which can disrupt foraging insects. They found that pest outbreaks of herbivorous insects occurred less in forests of mixed broadleaf and conifer tree species than the single tree areas. Further research is required to fully understand the influence of naturally occurring

non-host cues and of such treatments that can minimise damage of logs, wood product and seedlings within plantation forests.

In summary future research should consider;

- The incorporation of visual and olfactory non-host stimuli into the development of methods to limit damage from wood borers and bark beetles to plantation timber in high risk situations.

- The use of visual and olfactory cues of host and non-host attractants and repellents, and the potential use of light stimuli, for the protection of forest products (logs, timber or seedlings).

- The consideration of applying mixed forest strategies into plantation forestry, to utilise visual and olfactory barriers created by the presence of non-host broadleaved species within conifer forests.

Further study in the areas discussed should result in a greater understanding of ways to manage the bark beetles, *H. ater, H. ligniperda* and the longhorn beetle *A. ferus* in New Zealand. Through the current research, the understanding and management of forest insect pest problems in New Zealand has been advanced, as has our understanding of interactions between wood borers and bark beetles and host and non-host stimuli. Future research in the direction of non-host and mixed forest interactions for such beetle species will benefit forestry practices in New Zealand, as well as contributing to our general knowledge in the fields of forest ecology and applied entomology.

5   ACKNOWLEDGEMENTS

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