Management of *Hylastes ater* (Coleoptera: Scolytidae) attacking *Pinus radiata* seedlings

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any University or Institute and to the best of my knowledge and belief the thesis contains no material previously published or written by another person except where due reference is made in the text of the thesis.

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ABSTRACT

This study investigates the biology and ecology of the black pine bark beetle, *Hylastes ater* Paykull, attacking young *Pinus radiata* D. Don, during the period 1994-1997.

Aspects of the life history and ecology of the black pine bark beetle were studied under laboratory and field conditions and results incorporated into the development of a pest management strategy.

H. ater has three distinct generations during the year, with mature adults emerging November-January, March-April, and August-September. Following emergence, adults cause extensive damage and often death to the seedlings, in their search for suitable breeding sites. *H. ater* has caused significant economic damage to second rotation plantations of *P. radiata* seedlings in the Plenty Valley of Southern Tasmania since 1990.

Physical, chemical and microbial aspects of the host selection and attraction behaviour of *H. ater* were investigated involving identification of several primary and secondary attractants. Physical aspects involved in the attack of *H. ater*, including bark thickness, depth of attack, spatial location, position and condition of host material were studied under field conditions. Chemical aspects included field experiments and laboratory bioassay of host related volatile compounds and a range of compounds which have been reported as sex attractants or repellents in or to other bark beetles. This included the isolation of volatiles occurring within adult beetles at different stages of their development and analysis of feeding beetles and *P. radiata* host material over a period of time. Bacterial, yeast and fungal species associated with adult beetles were isolated, identified and both field experiments and laboratory bioassays conducted to evaluate any potential role in beetle behaviour. Parasitic nematodes associated with *H. ater* were isolated and identified.

Results were examined with regard to past and current management practices and recommendations made for reducing the future pest status of *H. ater*.

Firstly, the control and management of *H. ater* must be based on reducing the incidence of attack on stumps, logging slash and seedlings. This can be achieved by eliminating the soil-bark interface and reducing the moisture content of slash and stumps by slashing or chipping practices. Secondly, thorough planning of harvesting operations should be conducted to ensure large amounts of slash do not build up in adjacent areas. Thirdly, monitoring of future second rotation planting coupes should be conducted prior to site preparation and establishment to determine the likely risk of *H. ater* attack.

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LIST OF ABBREVIATIONS

A	Australian
ACI	Australian Consolidated Industries
ANM	Australian Newsprint Mills
γ-BHC	gamma-benzene hydrochloride
CIBC	Commonwealth Institute of Biological Control
CSL	Central Science Laboratory
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
HP	Hewlett-Packard
IIBC	International Institute of Biological Control
IMI	International Mycological Institute
MEA	malt extract agar
MCH	3-methyl-2-cyclohexen-1-one
MCOL	1-methyl-2-cyclohexen-1-ol
MS	mass spectrometry
NIST	National Institute of Standards and Technology
ODW	oven dry weight
PDA	potato dextrose agar
PVC	polyvinyl chloride
SAB	sabouraud dextrose agar
SEM	scanning electron microscopy
SIM	selected ion monitoring
SPME	solid phase microextraction
TAF	triethanolamine formalin
TSA	tryptone soy agar

LIST OF UNITS

ac	acre
cm	centimetre
ft	foot
g	gram
h	hour
ha	hectare
Hz	hertz
kg	kilogram
km	kilometre
l	litre
m	metre
mg	milligram
min	minute
ml	millilitre
mm	millimetre
sec	second

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CHAPTER 1 INTRODUCTION

The Californian native *Pinus radiata* D. Don commonly known as radiata pine or monterey pine, is the dominant plantation species for softwood production in the Southern Hemisphere. Radiata pine plantations extend from Western Australia through South-eastern Australia including Tasmania (Neumann 1979). Within Australia radiata pine comprises 75% of the coniferous plantation estate and is continually increasing (Anonymous 1997). Tasmania has 76,130 ha of radiata pine plantation, comprising approximately 10% of the radiata pine plantations Australia wide (Anonymous 1995). The future goal of the Australian Government and forest industry is to treble the plantation area of both softwood and hardwood within Australia from 1.1 to 3.3 million ha by the year 2020 (Anonymous 1997).

Australian Newsprint Mills (ANM) is Australia's largest producer of newsprint. It has paper mills at Albury in New South Wales and at Boyer in Tasmania. Plantations of *P. radiata* are sourced to provide fibre for thermomechanical pulping. The annual production of paper from the Boyer mill is 400,000 tonnes valued at A$ 40 million per annum. ANM's plantation estate in Tasmania is 23,000 ha.

The main limitations to the growth of radiata pine include climatic, topographic and edaphic restraints (soil, water, nutrients etc.), natural hazards (wind, snow, hail, frost, drought, lightning, and wildfire), operational damage (site impairment and degradation), weed competition and pests and diseases (Lewis & Ferguson 1993).

A bibliography of all forest entomology research in Australia to 1988, was prepared by the Australian Forestry Council (Wylie & French 1991). Australian pine plantations have generally remained free of significant insect damage inspite of the adaptation to pine of many indigenous insect species and the introduction of several pest species (Minko 1961c, Neumann 1979, Ohmart 1980). The European wood wasp (*Sirex noctilio* Fabricius) in Tasmania and Victoria, and the five-

spined bark beetle (*Ips grandicollis* Eichoff) in South and Western Australia have been exceptions (Neumann 1979, Ohmart 1980).

The family Scolytidae belongs to the order Coleoptera and the class Insecta (Stark 1982) and comprise a large number of pest species which are regarded as the most serious pests in forest ecosystems (Coulson & Witter 1984).

Members of the Scolytidae are found worldwide and attack woody and herbaceous plants including coniferous and broad leaved trees, coffee, tea, sugar cane, fruit and legumes (Stark 1982). More than 6000 species of Scolytidae have been described from throughout the world (Wood 1982). All scolytid species complete their whole life cycle within the host so can tolerate a wide range of environmental conditions (Stark 1982).

The family Scolytidae is divided into two groups based on their feeding habits, namely ambrosia beetles which are wood borers and bark beetles which are phloem feeders (Stark 1982). Although Scolytidae is used as the family designation, within Australia bark beetles are placed in the family Curculionidae and designated as the subfamily Scolytinae (Lawrence & Britton 1991). Bark beetles are regarded by many forest managers worldwide as the most economically important group of forest insects (Ciesla 1988), and are among the most destructive agents in forests (Berryman 1974), with 53 species of bark beetles recorded on *P. radiata* worldwide (Neumann 1987).

Bark beetles can be divided into two categories namely primary tree-killing bark beetles which invade and kill healthy trees and secondary bark beetles which attack logs, stumps, freshly cut material, and damaged or stressed trees (Rudinsky 1962, Stark 1982, Wood 1982, Neumann 1987). The first group must overcome the hosts defences and cause tree death before they can successfully exploit the host for brood development. Primary tree-killing bark beetles chiefly include species in the genera *Dendroctonus* as well as some *Ips* spp. (Ohmart 1982). Most bark beetles belong to the second group attacking stumps and freshly cut, moribund or severely stressed trees (Ohmart 1989). Examples of secondary

bark beetles include species belonging to the genera *Hylastes*, *Hylurgus*, *Hylurgops*, and *Pityophthorus* (Ohmart 1982).

Three introduced bark beetles have become established in radiata pine plantations in Eastern and Southern Australia, namely the black pine bark beetle (*Hylastes ater* Paykull), the golden-haired bark beetle (*Hylurgus ligniperda* Fabricius), and the five-spined bark beetle (*I. grandicollis*) (Neumann 1987, Lawrence & Britton 1994). *I. grandicollis* has resulted in significant tree mortality and contributes to blue staining in sapwood (Eldridge 1983). Both *H. ater* and to a lesser extent *H. ligniperda* have proved to be a serious problem in second rotation radiata pine plantations with the death of seedlings and young trees in South Australia, Victoria and New South Wales (Swan 1942, Minko 1965, Neumann 1979, Eldridge 1983, Neumann 1987).

The cypress bark beetle (*Phloeosinus cupressi* Hopkins) and the smaller European elm bark beetle (*Scolytus multistriatus* Marsham) have also been introduced and are established in South-eastern Australia among ornamental cypress and elm trees respectively (Neumann 1987, Lawrence & Britton 1994). *S. multistriatus* is being monitored as it is the vector of *Ceratocystis ulmi* Buism. C. Moreau, the cause of Dutch Elm disease (French & Rosel 1975).

Adult bark beetles are small slightly elongate and cylindrical hard bodied insects, 1-9 mm in length, and brown or black in colour (Coulson & Witter 1984). The antennae are characteristically elbowed and possess a distinct club at the tip (Pedigo 1989).

The life cycle of all bark beetles consists of egg, larval, pupal and adult stages with several generations being produced per year (Neumann 1987). The life cycle can be divided into three general stages, host location, selection and colonisation; brood development; and dispersal (Coulson & Witter 1984).

The life cycle begins with an adult beetle locating a host (Coulson & Witter 1984). Initial discovery may involve perception of the host by olfactory cues termed primary host selection or via random searching or visual cues (Stark 1982, Coulson & Witter 1984, Neumann 1987).

As detailed by Stark (1982) and Neumann (1987), once a host is selected, bark beetles may produce pheromones which sometimes combine with either host produced volatiles or auditory signals to result in the attraction of large numbers of additional adults. The response of adults to pheromones with or without host produced substances is termed secondary host selection (Coulson & Witter 1984). Repellent pheromones may also be produced by bark beetles to discourage further colonisation (Coulson & Witter 1984).

In some bark beetle species, the females initiate the attack and in other species it is the males that attack first (Hadlington 1951). The condition of the host can influence colonisation and hence subsequent brood development.

Once the adults arrive at the host, one of the sexes initiates entry through the bark by chewing into the phloem region (Coulson & Witter 1984). Many polygamous species create a special nuptial chamber for mating while monogamous species tend to mate on the bark surface near the entry hole or in the tunnel in a "turning" niche (Stark 1982). A characteristic tunnel is then excavated termed an egg gallery (Stark 1982, Wood 1982, Coulson & Witter 1984). The tunnels or galleries created by bark beetles are very varied and often characteristic of a species with specialised holes to the outside enabling ventilation and thus successful exploitation of the host material (Stark 1982, Wood 1982, Coulson & Witter 1984). Eggs are laid into small lateral niches within the egg galleries in the bark (Neumann 1987). The eggs are small, oval to round, slightly elongated and white in colour (Hadlington 1951) with the number of eggs laid by bark beetles ranging from six to up to 300 in some species (Stark 1982).

The final phase of the host selection and colonisation stage involves the fate of the attacking adult population. The adults may die within the gallery by either natural enemy attack or natural causes. In other cases adults may leave the host, a process called reemergence, and colonise additional hosts.

Following oviposition, the eggs hatch and the larvae feed by creating larval mines often at right angles to the main gallery, wherein the larvae mature (Coulson & Witter 1984). Most bark beetles have three or four larval instar stages

(Coulson & Witter 1984). The larvae are thick bodied, legless (apodous) and may be cream or white in colour with a distinct sclerotised head and dark coloured mandibles (Hadlington 1951).

Pupation or transformation to the pupal stage takes place at the end of the larval gallery often in a special pupal cell (Wood 1982). The pupa is unprotected and white in colour, which darkens and after a period hardens (Hadlington 1951).

The final stage involves the emergence of adults from breeding or overwintering sites within the host tree (Neumann 1987). Dispersal is the activity of leaving one host and successfully locating another (Coulson & Witter 1984). Bark beetles are able to adapt to weather and climate and adjust their subsequent emergence (Stark 1982). Temperature has a major influence on emergence and dispersal with beetle flight being inhibited above and below a temperature threshold (Stark 1982).

Bark beetles attack both hardwood and coniferous trees, although most of the pest species are associated with commercially valuable softwood species of pine, fir, and spruce (Coulson & Witter 1984). Tree killing bark beetles are very rare in angiosperms even though many scolytid species are associated with hardwoods (Ohmart 1989). This subject was reviewed by Ohmart (1989) and two hypotheses were suggested to explain this phenomenon. Firstly, that the wound response of hardwood phloem is so complex that bark beetles have never been able to successfully colonise this material. Alternatively, the relative physiological costs expended by bark beetles attacking hardwood phloem are greater than the nutritive value obtained from the material.

With bark beetles, the host tree is partitioned in the sense that a particular species will occupy or utilise a limited part of the host, and it is not uncommon for several species to share the same host tree (Stark 1982).

Bark beetles possess highly sophisticated systems of communication based on chemistry and sound and have developed symbiotic and communal relationships with many organisms (Stark 1982). Stridulation or the production of

sound by friction is associated with stress, rivalry, aggression, greeting and courtship (Stark 1982).

Many associations exist between beetles and fungi, yeasts and bacteria and numerous workers have indicated that various microorganisms may play a number of different roles (Francke-Grosmann 1963, Graham 1967, Whitney 1982, Coulson & Witter 1984). These roles include location of hosts, aggregation, overcoming host resistance, adult and larval nutrition, and conditioning hosts for brood development (Stark 1982).

The black pine bark beetle, *H. ater* was discovered attacking and causing significant mortality in ANM's second rotation plantations of *P. radiata* seedlings in the Plenty Valley, Southern Tasmania, in 1990. *H. ater* has subsequently spread throughout the second rotation planting. A second but rarer nondamaging exotic species *H. ligniperda*, also occurs.

Adult *H. ater* beetles feed on the bark of the entire root structure, resulting in the death of *P. radiata* trees ranging from newly planted seedlings up to 4-5 year old trees. In the Plenty Valley infestations of *H. ater* steadily increased to outbreak levels with 10-90% seedling mortality being recorded from 1990-1996.

Sustainable production demands that the company develops cultural techniques to allow replanting of harvested *P. radiata* sites. Currently, the viability of second rotation sites is threatened by the seedling mortality following attack by *H. ater*. This situation has come about primarily by a change in the forest management practice of burning slash of harvested sites to one of leaving the slash on the soil surface in the interests of conservation of nutrients, soil microbes and invertebrates and soil structure. This change of management combined with continued harvesting including clear felling operations has provided a continuous supply of readily available breeding material. In addition warmer weather conditions since 1990 have been favourable for bark beetle development. It could not be concluded that the beetle attack was caused by stressed trees, and the most probable theory was that the high seedling mortality recorded was caused by the change in slash management technique.

The only available control options involve either the burning of harvest slash or the intensive use of insecticides. Neither option is environmentally sustainable. Alternatively, sites need to remain unplanted for 1-3 years to permit stump and slash decay.

The objectives of this investigation were to research, design, and develop an effective, economic, and environmentally sustainable pest management control system in order to minimise the current significant losses of seedling replants caused by *H. ater* in Tasmanian pine plantations which impact on future pulpwood supply. Ideally the program should ensure that burning of harvested slash and the use of insecticides is not required but at the same time avoiding a fallow period of more than eight months and any extension of a current pulpwood rotation of 15-20 years.

The following areas were investigated: biology and general ecology; physical, chemical and microbial aspects of host selection and attraction involving identification of primary and secondary attractants; taxonomic identity, parasitic status and potential use of nematodes infesting adult beetles. The impact of forest hygiene practices and practical control measures on beetle numbers was evaluated. A secondary aim of the project was to incorporate findings into the development of an effective integrated pest management program.

CHAPTER 2 GENERAL MATERIALS AND METHODS

2.1. LOCATION

All field experiments were conducted within ANM's second rotation *P. radiata* plantation in the Plenty Valley from 1994-1997. The Plenty Valley is located approximately 20 km South-west of New Norfolk, Southern Tasmania at 43°10' S latitude and 146°52' E longitude, 250 to 680 m above sea level.

Harvesting of the first rotation (15-20 year old) *P. radiata* trees began in 1988, with large coupes being cleared consecutively up until 1996. The second rotation plantation estate in the Plenty Valley comprises more than 2000 ha, with between 1250-1430 *P. radiata* trees planted per ha. Planting stock consists primarily of seedlings with some cuttings planted in a few coupes.

Mean annual rainfall of 700-900 mm is experienced. Mean daily temperatures for January and July range from 10.0-23.6°C and 1.5-10.9°C respectively.

2.2. TRAPPING METHODS

2.2.1. Introduction

Initially a standard uniform method was required to successfully and efficiently catch adult *H. ater* beetles. A method was sought which could be used to monitor numbers, to assay potential chemical attractants in field experiments and to gain easy access to life stages.

Numerous trapping devices have been designed and utilised in research and trapping programs for scolytid beetles throughout the world. These devices include window glass barrier traps (Chapman & Kinghorn 1955), pitfall traps (Bedard et al 1990, Rieske & Raffa 1993), various sticky traps (Gara 1967, Browne 1978), funnel traps (Lindgren 1983) and drainpipe traps (McLean et al 1987). Most trapping devices are expensive and labour intensive to maintain (Moser 1976, Furniss 1980, Lindgren 1983).

Four different traps were evaluated in field trials and included perspex barrier pane traps, cage traps and two types of sticky mesh traps, which could be assembled at a relatively low cost and maintained efficiently. The traps were compared to partially buried 60 cm *P. radiata* billets cut from 17-20 year old trees.

2.2.2. Materials and Methods

2.2.2.1. Description of the traps

The perspex barrier pane trap shown in Plate 2.1 consisted of a 60 x 45 cm perspex pane fitted into a slot in a 73 cm length of 10 cm diameter polyvinyl chloride (PVC) pipe with removable end caps. The PVC pipe was attached to a wooden framework of dimensions 63 cm long and 61 cm wide. The PVC pipe was half filled with water and a small quantity of detergent, to act as a surfactant. The removable end caps enabled the pipe to be emptied at regular intervals and the insects present removed. The traps were positioned on the ground with two freshly cut, untreated *P. radiata* billets lying half buried directly under the PVC pipe and parallel with it. These logs provided a source of attractants. The two pane traps were established approximately five metres apart, one facing north and the other east. This orientation was selected to assess the bearing of beetle movement and cover both directions of flight.

The square cage traps as shown in Plate 2.2 consisted of heavy gauge mesh, one large (20 x 20 cm) and one small (15 x 15 cm). Each cage had a strip of heavy duty sticky tape from both sides covered in 'Stick-Um' (Tangle-Trap Insect Trap Coating, The Tanglefoot Company, Grand Rapids, Michigan 49504 U.S.A). The cages were positioned over a freshly harvested, untreated *P. radiata* billet buried vertically in the soil with 1/4 of the log above the soil surface.

The mesh billet traps consisted of two freshly cut, untreated *P. radiata* billets buried horizontally half in the soil, with flywire mesh attached with drawing pins to the exposed surface and then covered with 'Stick-Um'.

Three tin cylinder traps were constructed from empty clean peanut oil tins with a diameter of 25 cm and a height of 40 cm. Two of the tins had both ends removed to create a cylinder, and the third tin had just one end removed so that a base remained. The two tin cylinders were placed over a vertically buried freshly harvested, untreated *P. radiata* billet and a circle of mesh secured over the top with an octopus strap. The top of the mesh covering the tins was covered in a layer of 'Stick-Um'. The third tin was treated the same except that the billet was completely enclosed due to it having a base. The base was left on one of the tins to prevent beetle entry from the bottom of the billet.

The control billets comprised logs of the same 60 cm length utilised in combination with the traps, and were cut from the same freshly harvested *P. radiata* tree. The control billets were buried horizontally, half in the soil.

2.2.2.2. Establishment and assessment of the traps

Three trials of the various trapping methods were established during November 1994 - January 1995 in an area of newly planted seedlings (planted the previous winter).

Trial 2.1 comprised two pane traps, two cage traps, two mesh billets and two control billets and was established for a period of three weeks.

Trial 2.2 comprised two pane traps, two cage traps, two mesh billets and two control billets and was established for a period of four weeks. The three tin cylinder traps were established for the last two weeks of Trial 2.2.

Trial 2.3 comprised two pane traps and three tin cylinder traps, and was established for a period of four weeks.

During each trial each trap was checked on a weekly basis and any *H. ater* and *H. ligniperda* adult beetles present removed, counted and sexed. At the completion of each trial, all bark was removed from the accompanying billets of each trap and all bark beetles were collected, counted and sexed. Because of the fundamental necessity to have some form of monitoring throughout the duration of this study the results and evaluation are presented at this time.

2.2.3. Results

The number of *H. ater* beetles collected from the various trapping methods during all of the trials are shown in Table 2.1 as well as the number of *H. ater* beetles that invaded the *P. radiata* billets in combination with each trap.

None of the artificial trapping methods were shown to be effective in trapping *H. ater* adult beetles. The total number of *H. ater* adult beetles collected from all traps during all the trials was only 158. This can be compared to the 3113 adult beetles collected from the billets associated with all of the traps, a 20 fold difference.

Traps and Billets	Total *H. ater*/trap	Total *H. ater*/billet	% *H. ater* in traps
North Pane Trap and Billet	59	269	18.0
East Pane Trap and Billet	74	762	8.9
Large Mesh Cage Trap and Billet	4	314	1.3
Small Mesh Cage Trap and Billet	4	120	3.2
Large Mesh Billet Trap and Billet	5	415	1.2
Small Mesh Billet Trap and Billet	3	257	1.2
Cylinder tin trap and Billet	4	44	8.3
Cylinder tin trap and Billet	2	18	10.0
Cylinder tin trap with base	3	N/A	N/A
Total *H. ater*	158	3113	4.8

Table 2.1. Total number of *H. ater* adult beetles caught on the various traps and collected from the billets corresponding to the traps (Appendix 1)

2.2.4. Discussion

None of the trapping methods evaluated were effective in capturing *H. ater* adult beetles with only 4.8% of the adult beetles being found in the traps. The majority of beetles entered the *P. radiata* billets directly. The adult beetles exhibit an exceptional ability to find the *P. radiata* host and enter the material directly.

As a result of this preliminary investigation it was decided that untreated, freshly harvested *P. radiata* billets of various lengths cut from 17-20 year old trees be used as the major monitoring tool (shown in Plate 2.3). Billet length for the various trials was chosen for ease of collection, handling, transport, establishment and subsequent investigation, and so that the minimum number of trees were

required to provide the appropriate number of replicates. Uniform *P. radiata* billets were used for the various trials, being free of branches and surrounded by undamaged bark except for the cut ends. The number of beetles or entry holes recorded were standardised by comparing billets on the basis of bark surface area. Bark surface area was calculated as equal to π x billet diameter x billet height.

2.3. STATISTICS AND COMPUTING

All data requiring statistical analysis was analysed using analysis of variance anova procedure of SAS v6.0.

All means and standard errors of the mean were calculated in Microsoft Excel v5.0, graphs were produced in CA Cricket Graph v1.0 and Microsoft Excel v5.0, and the word processing package Microsoft Word v6.1 was used in the compilation of this thesis.

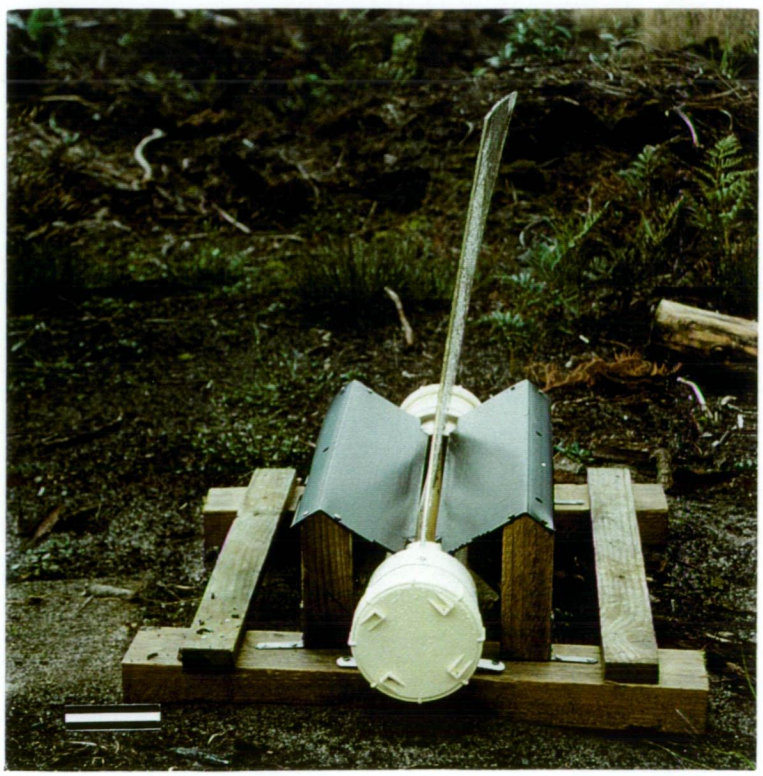

Plate 2.1. Perspex barrier pane trap (Bar = 10 cm)

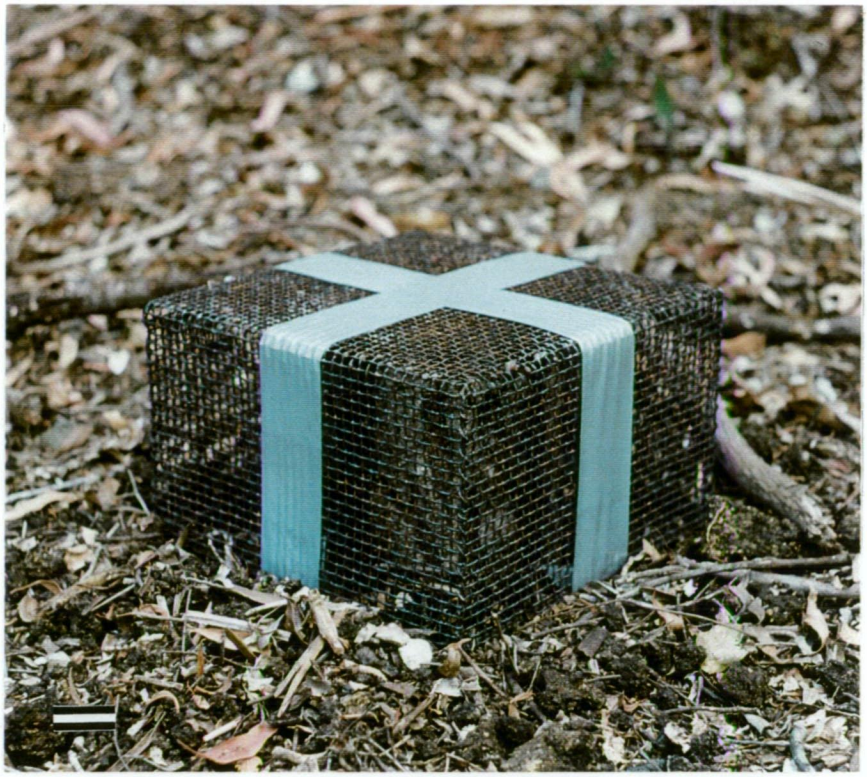

Plate 2.2. Cage trap (Bar = 5 cm)

Plate 2.3. Buried *P. radiata* billets used as the main monitoring tool (Bar = 10 cm)

CHAPTER 3 BIOLOGY AND GENERAL ECOLOGY

3.1. INTRODUCTION

H. ater attacks *P. radiata* stumps, logs and logging slash to feed but ultimately to provide breeding sites. During this process it provides a useful service by assisting in the breakdown of logging residues.

The significant problem with *H. ater* is the attack of *P. radiata* seedlings adjacent to stumps and logging slash. In their search for food and suitable oviposition sites the adult beetles feed on the bark of the entire root structure, either completely removing all the bark or forming galleries on the stem to the finest roots. The initial visual symptom of seedling attack is the wilting of the apical regions of seedlings progressing down the plant in time, with the ultimate death of the seedlings. The time from initial attack to seedlings displaying wilting and their eventual death may span several weeks to months, depending on the climatic conditions. It is not uncommon for more than 50 adult beetles to be present on the roots of a single one year old seedling.

No previous detailed studies of *H. ater* under Tasmanian conditions have been reported. Thus, before any management and control measures could be implemented the basic biology and general ecology of *H. ater* under Tasmanian conditions had to be determined.

Firstly, the life cycle of the black pine bark beetle was monitored under field conditions and in the laboratory with the use of *P. radiata* billets.

The sex ratio of adult *H. ater* beetles arriving at host material and the sex initiating entry through the bark needed to be determined to identify which sex initiates the attack on the host material in relation to host colonisation and selection mechanisms. The number and sex ratio of emerging adult *H. ater* beetles was also determined.

3.2. LITERATURE REVIEW

BIOLOGY, SEASONAL HISTORY, AND HABITS

3.2.1. General

3.2.1.1. Taxonomy

The original description of the black pine bark beetle was published by Paykull (Fauna Suec. Ins. 3, p.153, 1800). Minko (1962), gives the following brief general description of the adults: "The adult beetle is black in colour. It is 5.0-5.5 mm long and relatively easy to recognise by its thorax, which is approximately one-third of the length of the body. The small shiny head, which has a narrow keel between the eyes and mandibles, is equipped with a pair of small but well defined clubbed antennae. The tibiae of the legs are serrate, while the wing covers display shallow striae with numerous punctures".

3.2.1.2. Host trees

The black pine bark beetle is specific in its host selection to coniferous trees, and has been known to attack the stumps, logs, logging slash and seedlings of *Pinus* spp. (pine), *Picea* spp. (spruce), *Abies* spp. (fir), *Larix* spp. (larch), and *Pseudotsuga menziesii* Franco (Douglas Fir) (Milligan 1978). The black pine bark beetle most commonly attacks *Pinus* spp., and has been known to attack the following species:

P. radiata (Clark 1932, Eldridge 1983)

Pinus muricata D. Don (Clark 1932, Boomsma & Adams 1943)

Pinus nigra Arnold ex. Lawson (Eldridge 1983)

Pinus pinaster Ait. (Clark 1932, Boomsma & Adams 1943, Eldridge 1983)

Pinus ponderosa Douglas (Clark 1932, Eldridge 1983)

Pinus sylvestris L. (Eldridge 1983)

Pinus canariensis C. Smith (Boomsma & Adams 1943)

Pinus halepensis Mill (Boomsma & Adams 1943)

H. ligniperda attacks only similar *Pinus* spp. (Bain 1977).

3.2.1.3. Distribution

The genus *Hylastes* occurs widely in the Northern Hemisphere. *H. ater* is a native of Europe, being known from all European countries, from Spain to Russia (Swan 1942) as well as America, and a serious pest throughout England and Scotland (Burton 1968). *H. ater* was found in New Zealand in 1929 (Clark 1932).

Swan (1942) discovered *H. ater* in South Australia at Mt. Burr during 1937, and by 1942 it was also present at Penola. A survey commenced during October, 1960 by the Forestry Commission of New South Wales, found *H. ater* to be one of many insects attacking *Pinus* spp. (Moore 1962). The black pine bark beetle has been recorded from the New South Wales forestry regions of Bateman's Bay, Bathurst, Eden, Tumut, and Wagga Wagga (Eldridge 1983). Surveys conducted in North-eastern Victoria during 1972-73 and 1976-77, found *H. ater*, as the only economically important pest species present (Neumann 1979). *H. ater* was noted as being very common in pine plantations in the Australian Capital Territory in 1974 (Oswald 1976).

The secondary species, the golden-haired bark beetle, *H. ligniperda* is present in the same countries as *H. ater* but has been introduced into Japan, South Africa, South America and Sri Lanka (Cielsa 1988, Faulds 1989).

3.2.2. Life History

The life cycle of the black pine bark beetle consists of egg, larval, pupal and adult stages. The parent female beetle deposits the eggs in small lateral niches alongside the egg gallery in the inner bark. The eggs hatch, and the larvae feed by creating larval mines, wherein the larvae mature, and reach pupation in special chambers, where transformation to the adult stage takes place (Neumann 1987). The life history of the secondary species, *H. ligniperda,* is equivalent to that of *H. ater*, except where otherwise stated.

3.2.2.1. Adult beetle

Newly emerged adults are reddish brown in colour, but darken to black as they mature (Milligan 1978). The relative size of the beetle ranges from approximately 3.0-5.5 mm. Records show the following variation: 3.0-4.0 mm (Boomsma & Adams 1943), 3.5-4.0 mm (Clark 1932), 4.0-5.0 mm (Hadlington 1951, Milligan 1978, Eldridge 1983) and 5.0-5.5 mm (Minko 1962). Adults of *H. ater* feed in the inner bark of stumps, logging slash, and logs; and also the bark around the root collar of seedlings (Swan 1942, Bain 1977, Milligan 1978, Neumann 1987, Ciesla 1988). Adult beetles are strong fliers (Clark 1932, Swan 1942) with their mode of dispersal described as migratory swarming (Minko 1962).

The golden-haired bark beetle resembles *H. ater* so could easily be confused with it, although *H. ligniperda* adults are about twice the size of *H. ater*, browner and more hairy (Boomsma & Adams 1943). The adults are 6 mm long, 2 mm wide, with the surface bearing yellowish hairs (Milligan 1978).

3.2.2.2. Attack of stumps, logs, and logging slash

H. ater adult beetles attack *P. radiata* material following both clear felling and thinning operations (Clark 1932, Swan 1942, Boomsma & Adams 1943, Hadlington 1951). The attack of stumps, logs and logging slash is to feed but ultimately to provide breeding sites (Swan 1942).

3.2.2.2.1. Character of bark selected for attack

Boomsma and Adams (1943) observed that *H. ater* occurs more frequently under thick than thin bark (no dimensions given). *H. ater* favours large diameter thick-barked *P. radiata* logs (Neumann 1987), especially for breeding (Swan 1942). Breeding sites are found in stumps, logs and branches but *H. ater* is also able to breed in smaller sized material less than 10 cm in diameter (Scott & King 1974). Small stumps become suitable for breeding sooner than larger stumps, but conversely they remain suitable for breeding for a much shorter period (Scott &

King 1974). Pine bark becomes suitable for *H. ater* attack some period after felling, and then reaches a stage where it is unattractive, with bark more than a year old being unsuitable for *H. ater* (Swan 1942). Boomsma and Adams (1943) describe felled logs on the ground being attacked after three to four weeks, and on one occasion the invasion of a log after only a period of 10 days. In South Australia if beetles are present and climatic conditions are suitable, then host material is attacked immediately after felling (D. Boomsma, pers. comm.).

3.2.2.2.2. Location of attack

Breeding and feeding sites are usually found beneath the bark of fresh stumps, felled logs, and slash. Proximity to the soil appears to be a requirement for *H. ater* attack (Bain 1977, Milligan 1978, Eldridge 1983, Neumann 1987), favouring the log surface in direct contact with the soil (Clark 1932, Zondag 1982). Beetle entry is made at bark surfaces which touch the soil (Swan 1942). Evidence of attack is the presence of red frass, which is expelled from adult galleries (Swan 1942, Milligan 1978). Minko (1958) described the attack of *H. ater* on north-west slopes one month earlier than on south-east slopes presumably due to temperature effects.

3.2.2.2.3. Density of attack

Records from Mt. Burr, South Australia in 1943 showed a concentration of 10 to 20 adults to the square inch of infested areas of logs (approximately one to three adults per cm^2) (Boomsma & Adams 1943). In New Zealand, Milligan (1978) recorded groups of about 40 feeding adults present in a small log (no dimensions given). Records from the Ovens plantation, Victoria in 1956-57 detail a beetle population of 10,000/ac in an area where the slash residue was 600 ft^2/ac (Minko 1958) (This is equivalent to a beetle population of approximately 24,700/ha with slash residue of 16,000 m^2/ha).

3.2.2.2.4. Duration of attack

The duration of the attack ultimately depends on the suitability of the stump to support feeding and hence the brood population, with moisture content being the most important factor influencing suitability (Scott & King 1974).

3.2.2.2.5. Sex making initial attack

The sex of beetles making the initial attack needs to be clarified. It is known that male and female adult beetles meet in a nuptial chamber which is formed beneath the bark by the male, and that the female builds a single longitudinal egg gallery, with the male providing little assistance except for removing frass from the entrance (Eldridge 1983). Milligan (1978) describes the initiation of the gallery by the female. On the other hand Clark (1932) reported the involvement of both sexes in the construction of the egg laying tunnel, with the female proceeding first and the male clearing the frass. Aeration is ensured by the creation of specialised holes to the outside (Coulson & Witter 1984).

3.2.2.3. Egg galleries

The egg gallery is formed by the female adult in the inner bark. Milligan (1978) recorded the egg gallery as being 80-130 mm long, often roughly parallel with the grain of the wood. After the egg gallery is constructed, the female adult forms special egg niches, which are evenly spaced on either side of the gallery and, in which, the eggs are deposited.

3.2.2.4. Attack of seedlings

H. ater causes little or no damage during its immature stages, and was in the past considered to provide a useful service by assisting in the breakdown of logging residues (Minko 1958, Zethner-Møller & Rudinsky 1967). However, the adults may also feed on the soft bark near the root collars of seedlings and newly transplanted young trees causing their death (Clark 1932, Swan 1942, Minko

1961b, 1962). The adult beetles may also attack roots of mature trees (Minko 1961b).

H. ligniperda is also capable of attack on seedlings (Neumann 1987) and is more aggressive and abundant in areas where both beetle species are present (Cielsa 1988). Neumann (1987) also details the predominance of *H. ligniperda* over *H. ater* when they both coexist.

3.2.2.4.1 Location of attack

The adult beetles feed on the bark from the soil level downwards, either completely removing all the bark or forming galleries on the stem to the finest roots (Neumann 1987). In contrast, *H. ater* has also been observed attacking host material above the ground (Clark 1932, Boomsma & Adams 1943).

3.2.2.4.2. Density of attack

Up to 12 adult *H. ater* beetles have been discovered on a single seedling (Ciesla 1988). Milligan (1978) details seedlings supporting up to 15 adult beetles. Minko (1958) describes the presence of 70 adult beetles on two foot tall *P. radiata* natural regeneration. In Chile, eggs, larvae, and pupae of *H. ligniperda* have been found in 2.5 year old seedlings (Ciesla 1988).

3.2.2.4.3. Mortality due to attack

In 1942, at Mt. Burr, South Australia, 50% of *P. radiata* seedlings on five acres were killed due to *H. ater* attack (Swan 1942, Boomsma & Adams 1943). In 1957, in North-eastern Victoria *H. ater* feeding damage of *P. radiata* seedlings, was responsible for up to 18% mortality amongst groups of seedlings, but over-all mortality did not exceed 1% (Minko 1961b). In 1962, in the Stanley Plantation in North-eastern Victoria a 3.2 ha area of one year old *P. radiata* seedlings were killed by *H. ater* (Minko 1965). In Chile, the mortality rate is generally below 6%, but occasional losses of up to 65% have been experienced (Cielsa 1988). During 1985 large populations of *H. ligniperda* were experienced in South-eastern

Australia, resulting in the death of several hundred *P. radiata* trees (Neumann 1987).

3.2.2.4.4. Characteristics of seedlings selected for attack

The black pine bark beetle is capable of killing newly planted seedlings, up to 4-5 year old trees. In the Northern Hemisphere *H. ater* has been associated with the death of mature well established trees, 10-15 years old (Swan 1942). In 1957, in the Ovens Plantation, Victoria, 1% of seedlings aged 1-10 years were killed due to *H. ater*. This indicates older seedlings are susceptible, depending on the population pressure (Minko 1958). It has however been suggested that healthy seedlings are able to survive attack (Milligan 1978). Seedlings which are attacked and then killed tend to have malformed roots as a result of poor planting or bark injury caused by other insects or mammals (Cielsa 1988). Resistance to attack is often visible via the below ground parts of the seedlings being encrusted with resin (Milligan 1978).

Trees aged between four and 14 years have been subjected to damage by *H. ligniperda* (Neumann 1987).

3.2.2.5. Attack of cuttings

There has been no definitive work comparing the susceptibility of seedlings and cuttings to *H. ater* attack. A lack of references exist on the effect of the differences between seedlings and cuttings on susceptibility of plant material to beetle attack. Cuttings of *P. radiata* have generally thicker stems (7->10 mm) compared to seedlings (5-8 mm) (S. Hetherington, pers. comm.), and may have a more rigid root system (Mason & Trewin 1987). Cuttings are physiologically older than seedlings.

During the early stages of growth several workers have shown that seedlings are more susceptible and exhibit greater degrees of infection compared to cuttings, to various fungal diseases including needle blight, *Dothistroma* spp. (Power & Dodd 1984, Burdon & Bannister 1985, Carson 1988, Ades & Simpson

1990) and western gall rust, *Endocronartium harknessii* Hiratsuka (Power et al 1994). Power and Dodd (1984) showed that two year old seedlings were more susceptible and had much higher level of infection of *Dothistroma pini* Hulbary compared to rooted cuttings of a more advanced maturation state. Ades and Simpson (1990) also found a higher infection level of *Dothistroma septospora* Dorog. Morelet amongst seedlings compared to cuttings. This difference has been attributed to the greater maturation age of the cuttings compared to the seedlings.

Although, seedlings have been found to be more susceptible to disease than cuttings, this may not always be the case. Within a two ha block in New Zealand mortality during the first six years after planting was higher among cuttings than seedlings, and more deaths were experienced from Armillaria root rot among cuttings than seedlings (Klomp & Hong 1985). The influence of genetics, however, has never been discussed in any of the above comparisons.

Investigations by Brown (1974) noted no significant difference after three years establishment between the stem and root development of cuttings from *P. radiata* trees aged one, two, three and seven years and that of *P. radiata* seedlings.

3.2.2.6. Re-emergence of parent adults

When the stumps and slash are no longer suitable, parent adults are forced to emerge and seek new breeding and feeding sites.

3.2.3. Life Stages

3.2.3.1. Egg stage

A general description of the eggs of the black pine bark beetle has been given by Boomsma and Adams (1943) as "the eggs are ovoid, white in colour, 0.9 mm long, just visible to the naked eye". Milligan (1978) describes the eggs as less than 1 mm long, 0.4 mm wide, smooth, shining, rounded at the ends with nearly parallel sides.

3.2.3.1.1. Number of eggs

Eggs are laid in special tunnels constructed for the purpose, in the inner bark adjacent to the cambium. Over 100 eggs are laid in the individual notches which the female cuts in the lateral walls of the egg gallery (Clark 1932, Boomsma & Adams 1943, Minko 1958, Milligan 1978). The only recorded data of the number of eggs per unit area is from the Ovens Plantation data where Minko (1958) recorded 23 eggs per inch of a six inch egg gallery (approximately nine eggs per cm of a 15 cm egg gallery).

3.2.3.1.2. Incubation period

The incubation period of black pine bark beetle eggs was found to be from two to three weeks (Munro 1916, Clark 1932, Boomsma & Adams 1943).

3.2.3.2. Larval stage

The technical description of larval characters given by Beaver (1970), and particularly of the last instar, are too long to be quoted here. In general appearance, the larvae are transparent and glossy following eclosion from the egg. The larvae then turn white, except for the head which becomes yellow-brown in colour being heavily chitinised with dark coloured mandibles (Minko 1962). The larvae may reach 6-8 mm in length and 3-4 mm thick (Boomsma & Adams 1943). The mature *H. ater* larva is white, opaque, legless, cylindrical, slightly curved, being 5-6 mm long and 1.5 mm wide (Milligan 1978).

3.2.3.2.1. Larval mine

The young larvae feed on the inner bark forming their own lateral tunnels spreading out from the egg laying tunnel, which become more irregular as the tunnels develop until all the bark may be destroyed (Clark 1932). The galleries increase in size as the larvae develop and the wood may be engraved slightly where the bark is thin.

3.2.3.2.2. Larval instars

As the larvae grow they go through periodic moults during which both the soft larval skin and the hard chitinised head capsule is shed. Milligan (1978) concluded that the black pine bark beetle passes through four instars during its larval development, with the last instar involving the longest time period. The developmental rate of larvae is dependant on temperature (Milligan 1978). Clark (1932) and Boomsma and Adams (1943) found that the larvae fed for six to seven weeks and then entered a resting stage which varied from one to two weeks in summer to much longer over the winter.

3.2.3.2.3. Growth requirements

In the Ovens Plantation, Victoria, during 1957, 50% of the galleries present under thin bark dried out during hot weather, before the larvae could reach maturity (Minko 1958). Weather conditions play an important role as a regulating factor in reducing the population. Competition for food amongst larvae is also regarded as a major factor limiting numbers of survivors.

3.2.3.3. Prepupal stage

Before pupating the larvae undergo a resting stage, the length of which varies with the seasonal conditions. The larvae pupate at the broader ends of their tunnels, where a pupal chamber is formed.

3.2.3.4. Pupal stage

The pupa is white in colour, with small spikes on the head, thorax and abdomen, being a free and active pupa (Minko 1962). The pupa darkens just prior to the emergence of the adult (Boomsma & Adams 1943).

3.2.3.4.1. Duration of pupal stage

The pupal stage has been found to be quite short unless hindered by long periods of low temperature, although the duration varies less with the season than

that of other immature stages (Milligan 1978). Clark (1932), Boomsma and Adams (1943) and Milligan (1978) found the duration of the pupal stage to be 8-12 days, 8-10 days and 6-14 days respectively.

3.2.3.5. Pre-emergence period

The adult beetles may remain within the tunnels for some period before emerging as the bark may still be sufficiently suitable to enable feeding (Swan 1942, Milligan 1978). Also, parent beetles may continue to feed in the bark of the breeding material to recuperate their reproductive capacity and may then proceed to produce a second brood in the same material (Scott & King 1974).

3.2.4. Host Colonisation

All bark beetles progress through a series of sequential steps, during the period of colonisation of the host. Host colonisation selectivity is based on the host tree species and the physiological condition of the tree. Neumann (1987) has observed the colonisation procedure to consist of four stages: Emergence and dispersal of adults; Host selection; Aggregation; and Establishment of the brood.

3.2.4.1. Emergence

The adult beetles emerge and disperse from their breeding site to new susceptible hosts, or a reinvasion of suitable previously attacked hosts can occur. Emergence has been shown to be influenced by temperature. In the Northern Hemisphere, the threshold air temperatures for emergence from bark for bark beetles approximates 10°C and for flight dispersal 17°C (Neumann 1987).

3.2.4.2. Host selection

The selection of hosts may be the response to host produced attractants known as primary attraction or a response to visual cues or as a result of landing randomly on slash, stumps, logs, or seedlings or a combination of mechanisms. Primary attraction has been exhibited by two *Hylastes* spp., *Hylastes gracilis*

LeConte (Miller et al 1986) and *Hylastes nigrinus* Mannerheim (Borden 1977). Vite' and Pitman (1967) describe that *Hylastes* spp. respond to plant produced attractants in the form of volatile host materials although example compounds are not listed.

3.2.4.3. Aggregation

After bark penetration and feeding, the initial insects may produce attractant pheromones. The action of the pheromones with the host produced attractants, and visual cues, results in large numbers of adult beetles being attracted (Coulson & Witter 1984). This is known as secondary attraction.

In some species the number of adult beetles arriving on the host is regulated by the production of additional volatile compounds which mask the pheromones and host attractants, and are known as antiaggregation pheromones or inhibitors (Coulson & Witter 1984).

3.2.4.4. Mating and establishment of brood

Brood establishment then follows and involves mating, egg gallery formation, and oviposition. Monogamy (having only one mate) has been proposed to exist with the black pine bark beetle (Coulson & Witter 1984, Boomsma & Adams 1943, Minko 1958). Clark (1932) describes *H. ater* as monogamous with a single mating. Mating may take place in the gallery or in a special cavity known as the nuptial chamber (Coulson & Witter 1984).

3.2.4.5. Total brood period

In New Zealand the development from egg to adult has been found to take from 60 to 300 days (Milligan 1978). Zethner-Møller and Rudinsky (1967) detail work by Pfeffer (1955) in which *H. ater* requires two years for complete development (under conditions present in central Europe), with larvae hibernating during the first winter and adults the second. The rate of development of the brood varies according to weather conditions and soil temperature which in turn

varies according to the type of soil (Scott & King 1974). In North-eastern Victoria, the mature adult stage appears August-September, December-January and February-March, which is consistent with three distinct generations per year (Minko 1961a, 1962). Three generations per year is also experienced in New Zealand (Clark 1932).

3.3. MATERIALS AND METHODS

3.3.1. Arrival of *H. ater* to *P. radiata* Material

Four trials were established under field conditions with freshly harvested *P. radiata* billets (40 cm in length and 15 cm diameter) during different seasons in the same year. The billets were buried vertically in the soil (to 2/3 of their length) and then monitored on a daily basis or after a few days for *H. ater* attack. The aim was to determine the time of arrival after harvesting and which beetle sex was arriving first at fresh *P. radiata* host material. Any beetles which arrived were collected and dissected to enable sex determination. This method of sex determination was subsequently modified by the method of examining the last abdominal sternite to differentiate beetle sex (see page 31).

Trial 3.1 comprised 12 *P. radiata* billets which were checked five days after establishment. Trial 3.2 comprised eight *P. radiata* billets which were checked 19 and 43 h after establishment. Trial 3.3 comprised five *P. radiata* billets which were checked seven days after establishment, with individual beetles and those present in pairs collected separately. Trial 3.4 comprised 12 *P. radiata* billets which were checked six days after establishment, with individual beetles, those present in pairs and those present in a mass collected separately.

Some beetles collected from these trials and on other occasions were weighed, to determine if there were any weight differences between beetle sex and time of year.

3.3.2. Entry and Construction of Egg Gallery

As a result of trials 3.1, 3.2, 3.3 and 3.4 showing that both male and female adult *H. ater* beetles arrive at host material simultaneously, the bark from five of the *P. radiata* billets was removed carefully with a chisel following three weeks establishment. Adult beetle pairs present in the egg galleries being constructed were collected noting the position of each beetle. The beetles were then sexed to determine the beetle sex responsible for egg gallery construction.

3.3.3. Larval Instars

H. ater larval stages were collected from the billets in which *H. ater* field life cycle and development were being monitored. Identification of larval instars was made using an occular micrometer. The head capsule width of each larval stage was measured after standardisation of the micrometer using an 0.01 mm stage.

3.3.4. Pre-emergent Beetles

Pre-emergent *H. ater* and *H. ligniperda* adult beetles were collected from *P. radiata* billets established under field conditions to determine the sex ratio of emergent beetles. Trial 3.5 and Trial 3.6 comprised three billets each. The billets had been monitored over a period of three months to check the progression of the life cycle. All adult beetles were collected from each billet, counted and sexed.

The average number of pre-emergent *H. ater* adult beetles present in *P. radiata* material was calculated by counting all newly developed brown adults within more than 20 *P. radiata* billets established under field conditions which had also been monitored over time to check the progression of the life cycle.

3.3.5. Field Life Cycle

Billets 40 cm in length were harvested from freshly harvested 17-20 year old *P. radiata* trees. The billets were buried vertically 2/3 within the soil. On a monthly basis for a period of three years approximately 20 billets were established

in a group at more than 20 sites throughout the plantation area. These sites were at an elevation of 400-550 m above sea level, covered all aspects, with coupe areas ranging from 3-200 ha, which were deemed to be representative of the study area encompassing recently planted coupes to thinned coupes (age 15-18 years). Each group of billets were monitored at intervals to examine the progression of the life cycle and to provide a time scale of development. The billets were used instead of stumps due to ease of examination and standardising initial beetle invasion and development and for comparison with other field trials.

3.3.6. Laboratory Temperature Development

3.3.6.1. Introductory laboratory trial

Two 20 cm long *P. radiata* billets were placed in a large plastic container (container dimensions 30 x 30 x 40 cm) half filled with soil. The container was sealed with a lid which contained eight 2 cm diameter circles of fine mesh to enable ventilation. The billets had been established in the field for one week to enable *H. ater* invasion then placed in the plastic container which was stored in the laboratory at room temperature. The billets were examined at intervals to monitor progression of the life cycle and to determine whether the development of all life stages was possible under laboratory conditions.

3.3.6.2. Laboratory trial

Following the successful development of *H. ater* in the introductory laboratory trial, the same method was employed but with replication. The aim of this trial was to quantify the effect of temperature on the development of *H. ater* life cycle stages.

Twelve freshly harvested *P. radiata* billets of 20 cm length were established in the field being buried vertically 2/3 in the soil. After seven days three billets were placed in each of four plastic containers (as outlined above) containing soil collected from the vicinity of establishment. All billets showed

beetle invasion with the extrusion of frass and presence of beetles around the base of each billet.

One of each of the containers was placed at 15°C, 20°C and 25°C in isothermal incubators (Contherm III). The temperatures were selected as a result of incubator availability and to enable *H. ater* development within time constraints. The remaining container was placed in the laboratory at room temperature of 21°C. The billets were examined at intervals to examine the progression of the life cycle. At each examination a section of the bark was removed and measured, with the life stages present collected and counted. Water was added to the soil in each container at intervals to maintain constant soil moisture.

3.4. RESULTS

3.4.1. Arrival of *H. ater* to *P. radiata* Material

The summarised results for Trial 3.1, 3.2, 3.3 and 3.4 of the arrival of *H. ater* beetles to *P. radiata* material are shown in Table 3.1. The arrival of *H. ater* to *P. radiata* material occurs within the first week of establishment. For the first three trials the sex ratio of male to female adult beetles arriving at fresh *P. radiata* material was 1:1. For Trial 3.4 there appeared to be more males to females present. When the male and female beetles collected from all the trials are added together, 51% of all beetles collected were male and 49% female, which is a sex ratio of approximately 1:1.

Hours/Days After Establishment	Trial Number	Total Number Beetles	Total Number Males	Total Number Females	Ratio Male:Female (%)
5 days	3.1	304	145	159	47.7:52.3
43 hours	3.2	55	31	24	56.4:43.6
7 days	3.3	33	16	17	48.5:51.5
6 days	3.4	96	59	37	61.5:38.5
TOTAL		488	251	237	51.4:48.6

Table 3.1. Arrival of *H. ater* adult beetles to *P. radiata* material (Appendix 2)

Beetle mass was shown to be significantly different when compared by date with a p-value of 0.0001, but only marginally significant when compared by beetle sex, and no statistical relationship was shown between date and beetle sex in terms of beetle mass (Appendix 3). Beetles caught during November weighed more than those caught December-January, and likewise May with mean weights ranging from 0.018 to 0.05 g respectively.

3.4.2. Entry and Construction of Egg Gallery

Male and female adult *H. ater* beetles can be differentiated easily by examining the last abdominal sternite, which eliminates the need to dissect beetles to determine sex. The sternite is less convex in male beetles with the presence of a distinct depression covered in hairs which is lacking in female beetles as shown in Plate 3.1 and 3.2 respectively.

When the billets were dissected three weeks after establishment, the egg galleries as shown in Plate 3.3 being constructed by the adult beetles were between one and 20 cm in length. The characteristic nuptial chamber or turning niche was evident as a distinct 'niche' on the side at the beginning of each gallery. The number of beetle pairs and the sex ratios are shown in Table 3.2, along with the beetle sex that was at the front of each gallery. The front of the gallery is referred to as the direction in which the gallery is being constructed away from the nuptial chamber/turning niche. As can be seen from Table 3.2 all of the adult beetles removed from the *P. radiata* billets were present in the galleries as distinct male/female pairs, with all female beetles occurring at the front of each gallery in all instances.

Billet Number	Total Number of beetle pairs	Beetle sex at front of each tunnel
1	4	4 Females
2	6	6 Females
3	11	11 Females
4	4	4 Females
5	4	4 Females
TOTAL	29	29 Females

Table 3.2. Sex of *H. ater* initiating entry into *P. radiata* material

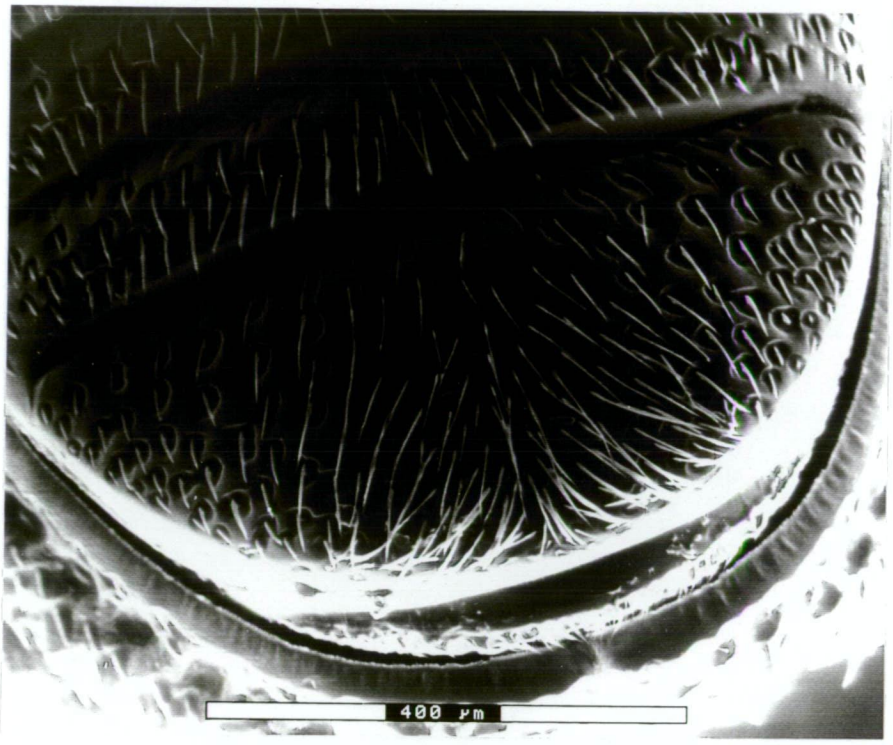

Plate 3.1. Abdomen of male *H. ater* adult beetle (x 115)

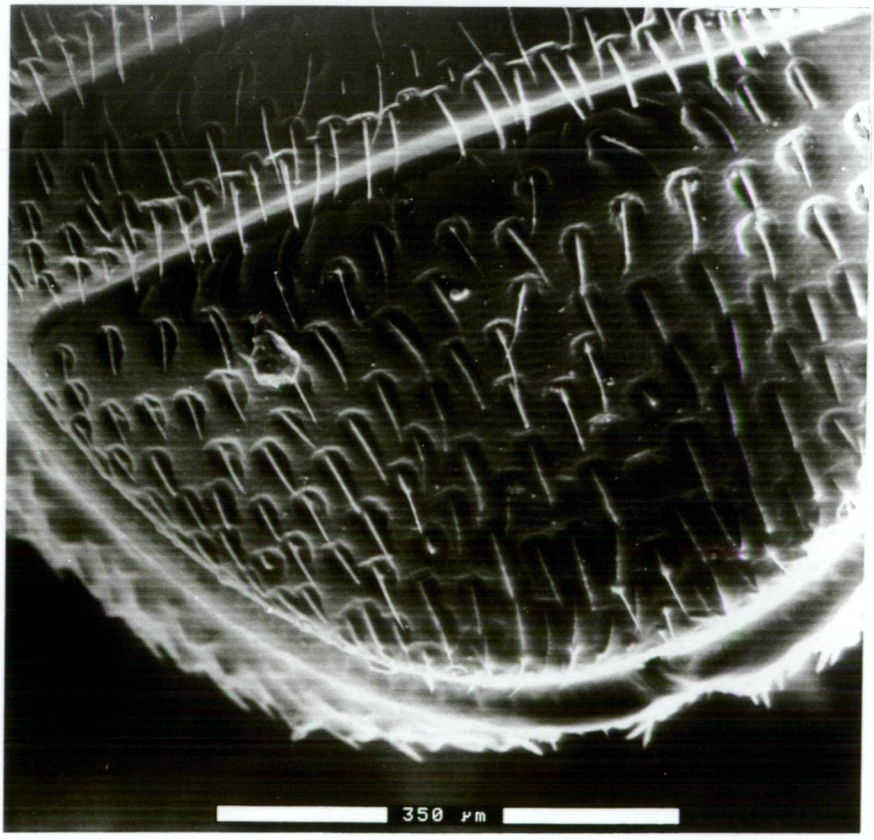

Plate 3.2. Abdomen of female *H. ater* adult beetle (x 130)

Plate 3.3. Egg gallery of *H. ater* (Bar = 5 cm)

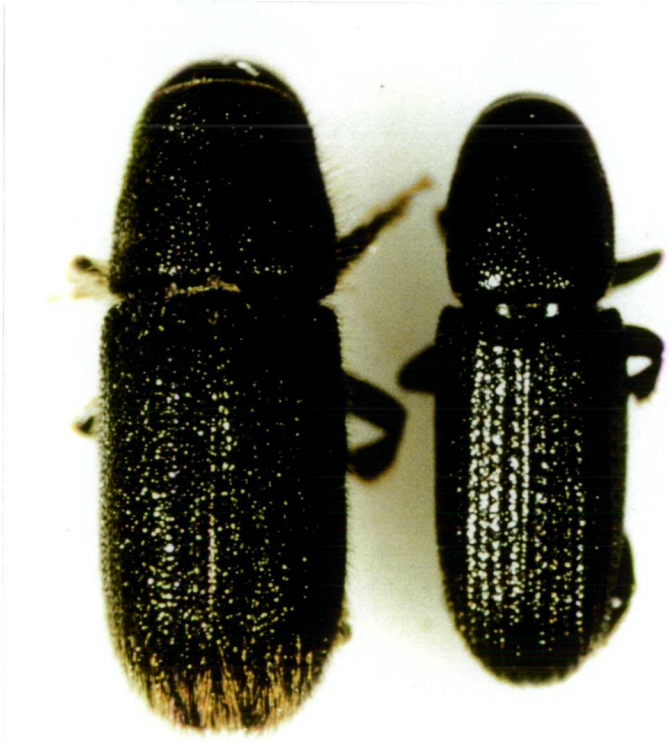

Plate 3.4. Adult bark beetles *H. ligniperda* (left) and *H. ater* (right) (actual length 6 mm and 4.5 mm respectively)

3.4.3. Larval Instars

The head capsule width measurements of 356 *H. ater* larvae collected from *P. radiata* billets are shown in Figure 3.1. This diagram shows the presence of four larval instars for *H. ater*. The mean and standard error of the head capsule widths of each larval instar is shown in Table 3.3. The means are estimated from the frequency peaks of the numbers of larvae measured. Mean head capsule widths for larval instars I, II, III and IV were 0.383, 0.512, 0.695 and 0.911 mm respectively.

Instar	Number examined	Mean ± SE
I	65	0.383 ± 0.019
II	108	0.512 ± 0.031
III	56	0.695 ± 0.049
IV	127	0.911 ± 0.064

Table 3.3. Mean head capsule widths of *H. ater* larvae collected on *P. radiata* billets (Appendix 4)

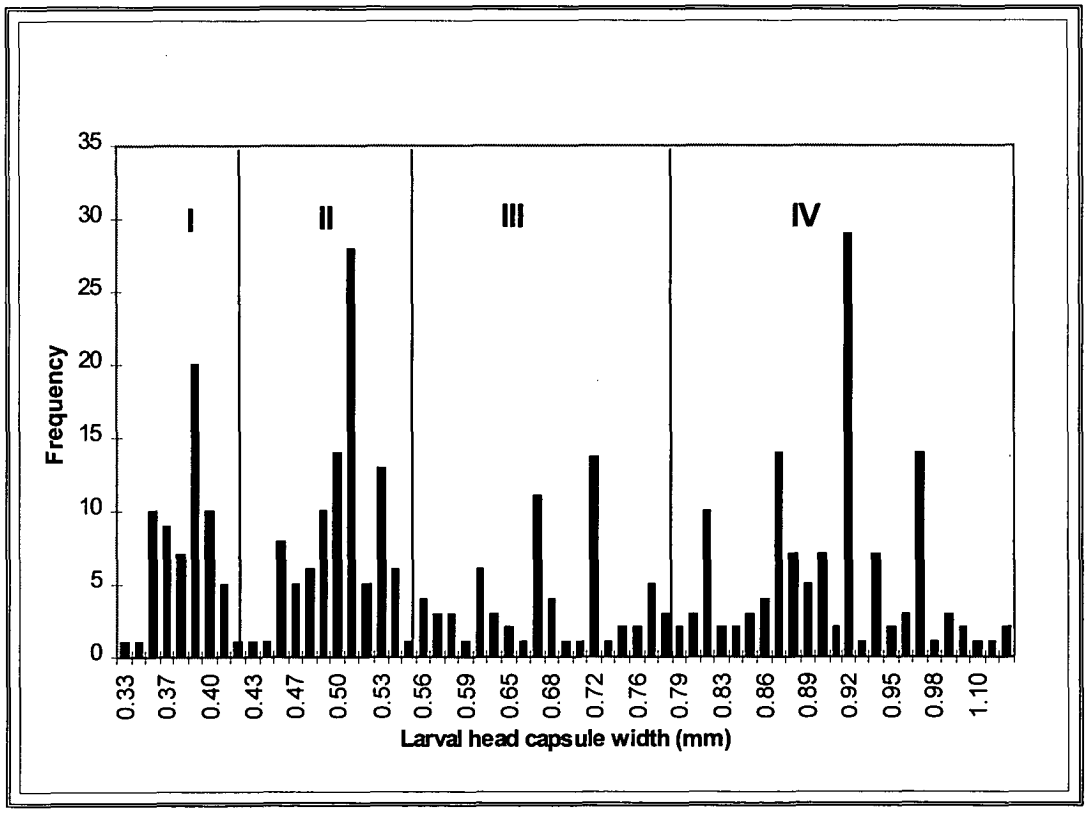

Figure 3.1. Head capsule widths of *H. ater* larvae (Appendix 4)

3.4.4. Pre-emergent Beetles

H. ligniperda was discovered in the Plenty Valley in association with *H. ater* during 1993. Adult *H. ligniperda* beetles are distinctly different to *H. ater* in size and characteristics as shown in Plate 3.4. Numbers of *H. ater* present within *P. radiata* material were always dominant over *H. ligniperda*. The average number of pre-emergent *H. ater* adult beetles present within *P. radiata* material was 790 beetles/m^2 of bark in contact with the soil. The maximum number of pre-emergent beetles present within *P. radiata* was found to be in the order of more than 4000 beetles/m^2 of bark in contact with the soil.

The number of male and female adult beetles of both bark beetle species found within the *P. radiata* billets is shown in Table 3.4. The sex ratio of male to female within host material is 2:1 for *H. ater* and 1:1 for *H. ligniperda*.

Trial	Total *H.ater*	Male *H.ater*	Female *H.ater*	Total *H.ligniperda*	Male *H.ligniperda*	Female *H.ligniperda*
3.5	445	293	145	123	70	53
3.6	190	126	64	81	31	50
Total	**635**	**419**	**209**	**204**	**101**	**103**

Table 3.4. Number of male and female *H. ater* and *H. ligniperda* adult beetles collected from established *P. radiata* billets

3.4.5. Field Life Cycle

The egg, larval, pupal and adult stages of the life cycle of *H. ater* are shown in Plate 3.5. Monitoring life cycle progression in buried *P. radiata* billets indicated that *H. ater* has three distinct generations per year as shown in Table 3.5. In Tasmania, mature adults emerge during November-January, March-April, and August-September. There will always be overlapping broods which do not conform to the general pattern. Newly emerged or callow adult beetles are light brown in colour which darken to black.

Adult *H. ater* beetles are found throughout the year invading *P. radiata* billets established in any month of the year although the most invasion occurs during March-April, August-September, and November-December-January when

mature adults emerge. Times of emergence correspond to noted times of seedling attack although damage can occur at any time during the year. Typical examples of seedling damage are shown in Plates 3.6 and 3.7. Larvae are also found year round with *H. ater* overwintering primarily in the adult and larval stages.

Adults emerge during August-September, eggs are found in August and September, and pupae in October, November and December. Eggs occur again in November-December-January, and March-April-May-June, while pupae are found again in January-February-March and August-September.

Jan	Feb	Mar	April	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
A		A	A				A	A		A	A
E		E	E	E	E		E	E		E	E
L	L	L			L	L		L	L	L	L
P	P	P					P	P	P	P	P

Table 3.5. *H. ater* life cycle progression (A=adult, E=egg, L=larvae, P=pupae)

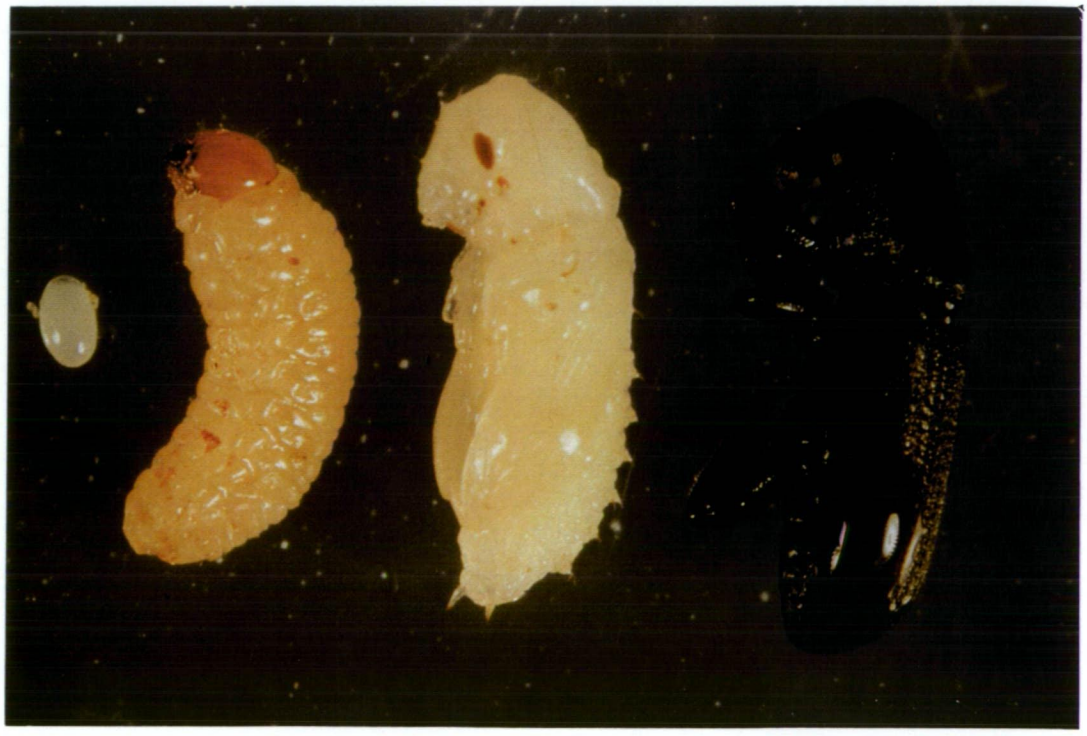

Plate 3.5. Life stages of *H. ater* - egg, larva, pupa, adult (L-R) (x 15)

Plate 3.6. First visual sign of *H. ater* attack (Bar = 5 cm)

Plate 3.7. Damage by *H. ater* to the roots of a one year old *P. radiata* seedling (Bar = 1 cm)

3.4.6. Laboratory Temperature Development

3.4.6.1. Introductory trial

The introductory trial billets were dismantled 84 days after initial beetle invasion or 77 days after establishment in the laboratory containers when the majority of the population had reached the adult stage as shown in Table 3.6. The use of the large sealed plastic container with adequate ventilation, under laboratory conditions, was thus suitable for *H. ater* development.

Billet	Larvae	Pupae	Brown Adults	Black Adults	Total Life Stages	Total Life Stages/cm^2
1	10	54	73	50	187	0.33
2	12	89	243	37	381	0.56

Table 3.6. *H. ater* life stages collected from laboratory established billets 84 days after beetle invasion

3.4.6.2. Laboratory trial

The number of days from initial *H. ater* invasion to the average maximum appearance of each developmental stage for each temperature treatment are shown in Figure 3.2 and Table 3.7. The quickest rate of development was recorded at the highest temperature of 25°C, with the majority of the *H. ater* population reaching the adult stage within 70 days of initial *H. ater* invasion. Even at the lowest temperature of 15°C the entire life cycle was completed within 115 days.

The highest temperature of 25°C caused the thinner bark of the billets to dry out with successful brood development partitioned to the thicker sections of bark. This problem was not experienced at the three lower temperatures with successful brood development in all billets and utilisation of all bark thicknesses.

In terms of day-degrees (d°C) *H. ater* development is summarised in Table 3.8, showing that 1791 day-degrees are required for complete development.

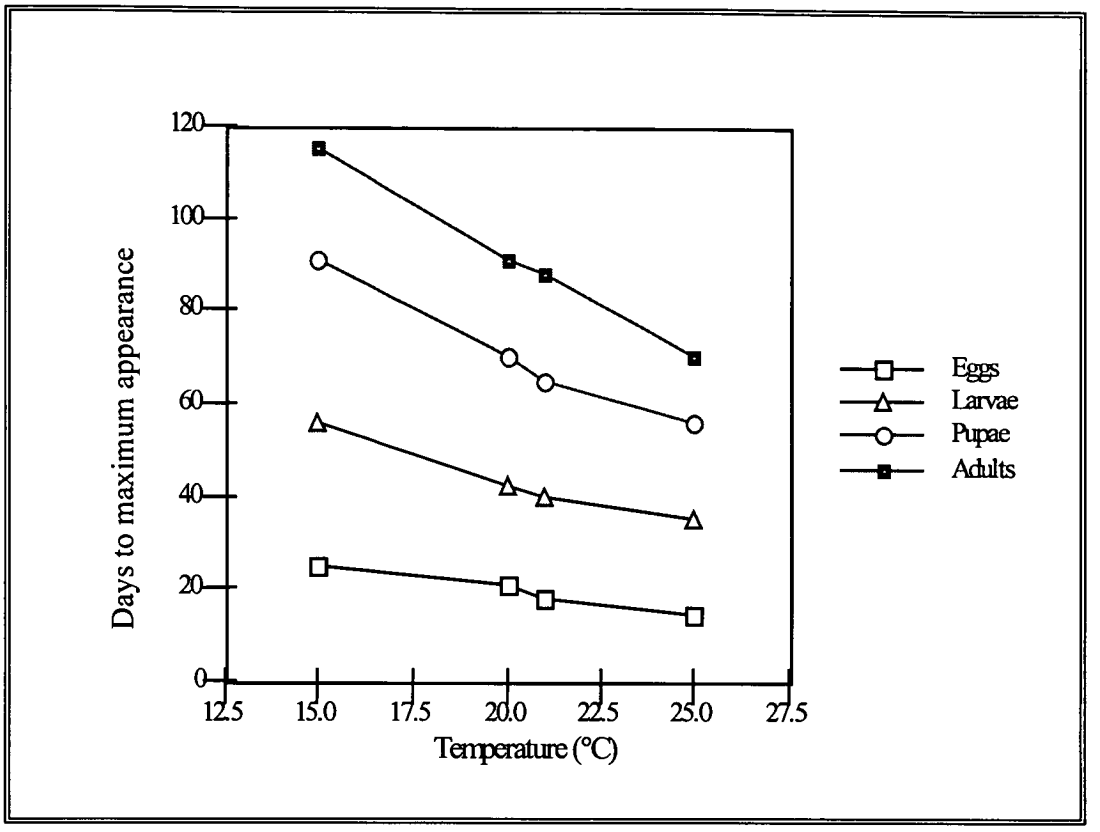

Figure 3.2. Laboratory development of *H. ater* at different temperatures

Temperature (°C)	Egg	Larvae	Pupae	Adult	Total Days
15	25	31	35	24	115
20	21	21	28	21	91
21	18	22	25	23	88
25	14	21	21	14	70

Table 3.7. Average duration (days) from initial *H. ater* invasion for maximum occurrence of each developmental stage

Temperature (°C)	Egg	Larvae	Pupae	Adult	Total d°C
15	375	465	525	360	1725
20	420	420	560	420	1820
21	350	462	525	483	1750
25	378	525	525	350	1869
Mean ± SE	381 ± 29	468 ± 43	534 ± 18	403 ± 62	1791 ± 66

Table 3.8. Development of *H. ater* in terms of day-degrees (d°C)

3.5. DISCUSSION

3.5.1. Arrival of *H. ater* to *P. radiata* Material

H. ater adult beetles arrive at fresh *P. radiata* material in large numbers within the first week of establishment as shown in the four trials with 485 beetles arriving at the *P. radiata* billets within the first seven days. This was substantiated in all other billet trials with the arrival of adult beetles to freshly harvested *P. radiata* billets within the first week of establishment. The only variation to this was sometimes found during periods of rain and low temperatures (< 5°C) especially during the winter months. For Trial 3.2 the *P. radiata* billets were established in the afternoon and when checked the following morning (19 h later) no beetles were present on the billets, but by the next morning (43 h later) 55 adult beetles were collected. This indicates that under suitable conditions *H. ater* adult beetles are capable of attacking *P. radiata* billets within two days of felling. Swan (1942) recognised that pine bark becomes suitable for *H. ater* attack some period after felling but the only time of arrival record is presented by Boomsma and Adams (1943) who found that felled logs on the ground were attacked after three to four weeks, and on one occasion the invasion of a log after only a period of ten days. However, this was only a casual observation and South Australian experience indicates that if environmental conditions are suitable attack occurs almost immediately (D. Boomsma, pers. comm.). It is assumed that the warmer climatic conditions in South Australia compared to Tasmania would make host material suitable earlier.

The sex ratio of adult *H. ater* beetles arriving at *P. radiata* material is equal even within the first 43 hours of *P. radiata* billet establishment. No previous work has been documented concerning the sex ratio of adult *H. ater* beetles arriving at *P. radiata* material. It appears that for *H. ater* no one sex is responsible for host selection with both male and female beetles simultaneously arriving at host material in a 1:1 ratio.

3.5.2. Entry and Construction of Egg Gallery

The female beetle initiates entry through the bark and a distinct niche is formed at the beginning of the gallery. The female plays an important role in host colonisation constructing the egg gallery with the male beetle following behind. These observations agree with those of Clark (1932) and Milligan (1978). When mating *H. ater* pairs were observed entering *P. radiata* material, the female always entered first. The male beetle following the female is indicative of playing an important role in defence of the female and subsequent egg gallery against predation.

Stark (1982) describes the formation of a special nuptial chamber for mating in polygamous bark beetle species while monogamous bark beetle species mate on the bark surface near the entry hole or in the tunnel in a 'turning' niche. *H. ater* beetles were observed mating on the bark surface prior to entry; while the female was initiating entry; as well as within the *P. radiata* material. The 'niche' formed by *H. ater* may be a nuptial chamber or a turning niche as described by Stark (1982). These results conflict with Eldridge (1983), who described the construction of a nuptial chamber by male *H. ater* beetles.

The differentiation of adult male and female *H. ater* beetles on the basis of the last abdominal sternite with the presence of hairs in a distinct hollow in male beetles (Munro 1916, Clark 1932), is a useful character, which eliminates the time consuming activity of dissecting beetles to determine sex.

3.5.3. Larval Instars

The only record of the number of larval instars which *H. ater* passes through during its larval development is presented by Milligan (1978) as four larval growth stages in New Zealand. However, no size distribution for the different instars was given. The same number of larval instars was found under Tasmanian conditions. The head capsule width of the last larval instar of 0.911 mm compares with that given by Beaver (1970) of 0.9-1.1 mm.

3.5.4. Pre-emergent Beetles

Large numbers of pre-emergent *H. ater* adult beetles were found within *P. radiata* material in contact with the soil. This demonstrated the ability of *H. ater* to build up to large populations under appropriate conditions and an indication of the numbers which emerge to find new hosts and in doing so cause damage to the surrounding seedlings.

In the Plenty Valley, *H. ater* was always predominant over *H. ligniperda*. *H. ater* was most often found as the sole occupant of *P. radiata* material, or if both species are present only small numbers of *H. ligniperda* were found compared to *H. ater*. Only on two occasions throughout the period of research was *H. ligniperda* found to be predominant over *H. ater*. The site characteristics on these two occasions were very similar. The sites comprised sandy soils which were subject to waterlogging. The *P. radiata* billets were dissected after a period of waterlogging. It is assumed that *H. ligniperda* is able to withstand such conditions. *H. ligniperda* adult beetles also tended to invade *P. radiata* material in which *H. ater* was vacating.

The dominance of *H. ater* in the Plenty Valley differs from the literature for mainland Australia which states that *H. ligniperda* is usually predominant over *H. ater* when both coexist (Neumann 1979, 1987, Cielsa 1988).

The sex ratio of developing *H. ater* beetles was calculated to determine if there was a relationship with the arrival of beetles to host material or the sex making initial attack. The sex ratio of pre-emergent *H. ater* beetles was 2:1 whereas that of *H. ligniperda* was 1:1.

3.5.5. Field Life Cycle

In the Plenty Valley, *H. ater* was found to have three distinct generations per year with mature adults appearing August-September, November-January, and March-April. This is consistent with *H. ater* records for Victoria (Minko 1961a, 1962) and New Zealand (Clark 1932).

3.5.6. Laboratory Temperature Development

The introductory trial demonstrated the successful rearing in the laboratory of *H. ater* from initial invasion through to new adult beetles. At room temperature *H. ater* was capable of developing through the entire life cycle within 84 days or 12 weeks.

The subsequent trial detailed the successful development of *H. ater* from initial invasion of *P. radiata* billets through to new adult beetles in 70, 88, 91 and 115 days (10, 12.5, 13 and 16.5 weeks) respectively at each temperature of 25°C, 21°C, 20°C and 15°C. The average number of day-degrees for the development of each *H. ater* stage of egg, larval, pupal and adult were determined to be 381, 468, 534 and 403 d°C respectively.

The time required for development from egg to adult fall within the range given by Milligan (1978) in New Zealand of 60 to 300 days. The rate of development increasing with temperature agrees with Milligan (1978) who recorded the duration of development being least during the warmer months and greatest during the cooler months of the year.

Investigation of *H. ater* development at 10°C in the laboratory would be recommended, as winter temperatures in Tasmania (and often autumn-spring temperatures) are below 15°C.

The influence of temperature on *H. ater* development impacts on brood development in thin bark, as shown by successful brood development at 25°C, being restricted to thicker bark sections of *P. radiata* billets. Although temperatures of greater than 25°C would generally not occur in Tasmania. Minko (1958) in Victoria also found that developing broods under thin bark dried out during hot weather. Temperature therefore plays an important role in determining successful brood development in thin bark.

CHAPTER 4

HOST SELECTION AND ATTRACTION - PHYSICAL ASPECTS

4.1. INTRODUCTION

Physical aspects involved in the attack of *H. ater* on its host substrate including bark thickness, depth of attack, spatial location, position and condition of host material needed to be investigated prior to the determination of any control strategy, in terms of the forest management change in slash management to one of slash retention.

Several slash treatments were trialed in 1996 by ANM to evaluate the effectiveness and economics of treating slash. The four treatments included retaining slash following harvesting procedures, removing all slash, breaking slash into small components with a slasher and moving over the slash with a spot cultivator to create clear holes at intervals for the planting of seedlings.

The slash components which were attacked by *H. ater* and the bark thicknesses invaded and required for the complete development of *H. ater* were undefined for Tasmanian conditions. It was also uncertain as to what depth *H. ater* could attack slash which was incorporated into the soil, and whether *H. ater* could search for completely buried material.

During preliminary investigations it was noted that the moisture content of the wood and bark appeared to influence the attractiveness of *P. radiata* billets to attack by *H. ater*. The effect of moisture content on the attractiveness of billets was investigated. Another aim was to determine if dry unattractive billets with very low moisture contents could become attractive if the moisture content was raised and, if so, determine the duration of attractiveness of wet billets. It was also necessary to determine how long the harvested stumps remain suitable for *H. ater* brood development. Practically such information could be utilised to predict how long harvested slash could remain attractive to *H. ater*, and suitable for brood development.

4.2. LITERATURE REVIEW

4.2.1. Physical Aspects of Host Material

H. ater tunnels, feeds and breeds under bark which is, or has been, in close contact with the soil (Clark 1932, Swan 1942, Milligan 1978, Zondag 1979, 1982). Adams (1950) details the presence of *H. ater* in the parts of slash in contact with the ground, or slightly embedded. Direct proximity to the soil appears to be a requirement for *H. ater* attack (Eldridge 1983).

Neumann (1987) describes the selection by *H. ater* of large diameter thick-barked logs in contact with the ground, or stumps and dead trees with thick bark on the root collars or the main roots just below ground level. *H. ater* is found more often under thick bark than thin bark (Boomsma & Adams 1943). Minko (1958) noted the preference of *H. ater* to breed in recently felled logs and stumps in shady, moist and sheltered sites.

Minko (1958) describes the drying out of thin bark during hot weather in the Ovens Plantation (Victoria) that resulted in the death of 50% of the developing *H. ater* brood. Only host material two or more inches in diameter (five or more cm) was subject to prolonged attack (Minko 1958).

Amman (1972) demonstrated a relationship between phloem thickness of lodgepole pine, *Pinus contorta* Douglas, and brood production of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins. There was a linear increase of emerging beetle numbers with phloem thickness when food supply was a limiting factor on brood production. Emerging beetle numbers increased exponentially with an increase in egg gallery length when food supply was not limiting (Amman 1972).

4.2.2. Slash Conservation

The practice of slash retention is being adopted worldwide due to the advantages of nutrient conservation and soil structure. Other advantages such as moisture conservation, weed management and temperature buffering have also

been associated with slash management (S. Hetherington, pers. comm.). Forest managers are recognising the implications of forest operations on long-term site productivity, focussing on maintaining and improving site productivity (Dyck & Beets 1987, McMurtrie 1997, Fleming et al 1998). The physical and chemical properties of soil are influenced by organic matter more than any other factor (Zwolinski et al 1993). Bark beetles play an important role in initiating the breakdown of logging residues ultimately contributing to organic matter.

The resulting residue from harvesting contains large quantities of nutrient elements (Smethurst & Nambiar 1990, McMurtrie 1997). Zwolinski et al (1993) detail the beneficial changes in surface and soil organic matter with the practice of slash retention. Burning volatilises some nutrients such as nitrogen and transforms others from organic to inorganic forms. These inorganic forms are more available to plants but are also more readily leached (Raison 1979). As detailed by Heal (1979), an important source of nutrients is present within logging slash and forest floor material, especially nitrogen. The logging slash and forest floor material contains a higher proportion of easily decomposed material than soil organic matter (Heal 1979).

The practice of burning has resulted in the loss of organic matter and nitrogen and thus productivity has declined (Keeves 1966, Farrell 1984, McMurtrie 1997). The loss of nitrogen through burning slash has been estimated to be 420 kg/ha (Lehane 1995) and up to 1000 kg/ha (Feller et al 1983). Increased availability of nitrogen was demonstrated with residue retention (Smethurst & Nambiar 1990). The significance of residue retention to productivity is dependent on soil type, existing nutrient reserves and availability (Farrell 1990, Smethurst & Nambiar 1990). As the number of rotations on the same site increases, the significance of residue retention will also increase.

4.2.3. Sound Production

Stridulation is defined by Barr (1969) as the production of sound by the "rubbing together of specialised structures on two body surfaces". Stridulation is

highly developed in members of the Scolytidae (Stark 1982) with stridulatory mechanisms present within 77 species in 23 genera (Barr 1969). Stridulatory organs appear to be present in the sex opposite to which initiates the entrance tunnel (Barr 1969, Stark 1982).

Three types of stridulatory structures are present in the Scolytidae described by Barr (1969) as vertex-pronotal, gula-prosternal, and elytra-abdominal tergites. Stridulatory organs consist of two parts a 'pars stridens' and a 'plectrum' (Barr 1969). Definitions of these components of the stridulatory organ has been quite confused as detailed by Barr (1969). The pars stridens is described as a file like series of ridges, spines, tubercles, teeth or other structures (Barr 1969). The pars stridens is often the more complex part which vibrates to produce sound. The plectrum is scraper like and may consist of a tooth, line of teeth, ridge, tubercle or other structures. The plectrum is regarded as the less complex part but is responsible for initiating the vibrating of the pars stridens.

As detailed by Stark (1982) stridulation has been associated with stress, rivalry, aggression, 'greeting', and courtship. Male stridulation results in the release of a pheromone by the female beetle in *D. ponderosae* and *Dendroctonus brevicomis* LeConte (Rudinsky et al 1976). Female *Ips confusus* LeConte are known to stridulate when entering the entrance tunnels constructed by the males (Barr 1969). Removal of the stridulatory organs of *I. confusus* females resulted in their entrance into the gallery being denied or delayed by the males (Barr 1969, Stark 1982).

Hylastes spp. are capable of stridulation (Marcu 1931). Listening to living beetles, Wichmann (1912) concluded that *H. ater* and *H. ligniperda* possessed sound apparatus on the tergites of the last segment of the abdomen and the underside of the elytra. The sound spot on the tergite, consists of four non fused segments symmetrically near the back edge of the beetle visible as a yellowish, less strongly chitinised, matt shine spot with no or less hairs (Wichmann 1912). A sound spot is also present on each side of the underside of the elytra, and sound is produced when the abdomen touches the wing covers (Wichmann 1912).

Munro (1916) describes the presence of a 'rudimentary' stridulating organ on the seventh tergite of male *H. ater* beetles consisting of "two tiny processes on the posterior border of the tergite and a stouter chitinous band extending across the tergite just anterior to these processes". Both tergite seven and eight are visible on male *H. ater* beetles with the seventh tergite covering the eighth tergite on female *H. ater* beetles (Munro 1916).

Marcu (1931) describes the presence of a stridulatory organ on the underneath of the elytra known as the pars stridens in both *H. ater* and *H. ligniperda*. The structure consists of a series of parallel strips at right angles to the edge of the elytra between the edge and the second row of dots. The distance between the parallel strips is even for *H. ater* but not the same for *H. ligniperda*.

Barr (1969) summarises the early work of Wichmann (1912), Munro (1916) and Marcu (1931) detailing the production of sound by male *H. ater* adult beetles with an elytra-abdominal tergite type of stridulatory structure. This type of stridulatory structure consists of a pars stridens as a series of parallel, transverse ridges on the undersurfaces of the elytra near the apices and the sutural margin; and a plectrum located on the abdominal tergites (Barr 1969).

4.3. MATERIALS AND METHODS

HOST ASPECTS

4.3.1. Substrates Attacked

Second rotation areas where slash had been treated in four different ways (slash retention, complete slash removal, mechanical slashing and spot cultivation) were surveyed and sampled to determine the treatment effects on *H. ater* abundance and provision of potential breeding material. The first three treatments involved pushing slash aside for routine ripping and mounding for line planting. The fourth treatment involved spot cultivation with a specialised cultivation head on an excavator. The areas were surveyed three months after the various slash

treatments were performed. The diameter and bark thickness of all slash items either suspended above, on or within the soil and the incidence of attack on each slash item was recorded. For each of the four treatments twelve 1 x 2 m areas were selected at random and analysed in detail over each treatment site.

4.3.2. Bark Thickness

The effect of bark thickness on *H. ater* was studied under Tasmanian field conditions using *P. radiata* billets of different diameters and bark thicknesses. Three similar sized *P. radiata* trees (comprising trials 4.1, 4.2 and 4.3) were felled and cut into 60 cm long billets from the base to the apex. Small discs approximately five cm wide were cut between each billet, and returned to the laboratory where the bark and phloem thickness, and total diameter of the discs were determined with calipers. Thus, the bark thickness of each billet was the average of two discs, one from each end of the billet. In the same way, the moisture content on the basis of oven dry weight of the bark was determined by averaging three samples taken from each end of the discs.

Individual freshly harvested billets were buried vertically with 40 cm below and 20 cm above the soil surface. Billets 60 cm in length buried with 20 cm above the soil surface, were chosen to enable more of the billet below the soil surface compared to previously used 40 cm long billets, and 20 cm was deemed to be an average stump height in the second rotation plantation. The billets of trial 4.1 were left for eight weeks, then lifted, bark removed and the number of adult beetles recorded. The billets from the other trees (trials 4.2 and 4.3) were left for 12 weeks then lifted, bark removed and numbers of developmental stages recorded.

4.3.3. Depth of Attack

4.3.3.1. Depth of attack trial 4.4

Twenty 60 cm long billets were cut from a freshly harvested, 17 year old *P. radiata* tree. The billets were buried vertically at four different depths in relation

to the soil surface, namely the top of the billet 20 cm above the soil surface, at soil surface, then 20 cm and 40 cm below the soil surface. Five replicates of the four burial depths were employed. Billets were left undisturbed for three months and then removed from the soil and examined following the removal of the bark. The extent of *H. ater* attack was determined by recording the number of individual galleries.

4.3.3.2. Depth of attack trial 4.5

Two similar *P. radiata* trees were cut into duplicate billets ranging in length from 30 to 100 cm respectively by 10 cm increments. One set of the eight billets acted as controls and were buried vertically with 20 cm above the soil surface. The other set of eight billets were covered in ACI aluminium sisalation paper as shown in Figure 4.1 and then buried vertically with 20 cm above the soil surface. The sisalation paper was cut to the appropriate length and wrapped around each billet and secured with heavy duty tape.

All billets were left for two and a half months and then lifted and the bark removed to determine the extent of *H. ater* attack by counting the number of individual galleries.

The aim of the trial was to determine at what depths *H. ater* could attack slash which was incorporated into the soil and if *H. ater* would search for suitable entry sites if prevented from entering by the sisalation paper.

4.3.3.3. Depth of attack trial 4.6

A 17 year old *P. radiata* tree was cut into eight 80 cm billets. Two control billets were buried vertically, one billet with 20 cm above the soil surface and the end of the other billet buried level with the soil surface. The remaining six billets were treated with sisalation paper as shown in Figure 4.2, with two replicates of each treatment A, B, and C. As this was only a preliminary trial only two replicates were used. The billets were left for a period of approximately four months, and then all of the billets were lifted and the bark removed to examine for

H. ater attack. The aim of this trial was to determine whether a visual cue combined with a chemical stimulus is involved in the host selection process.

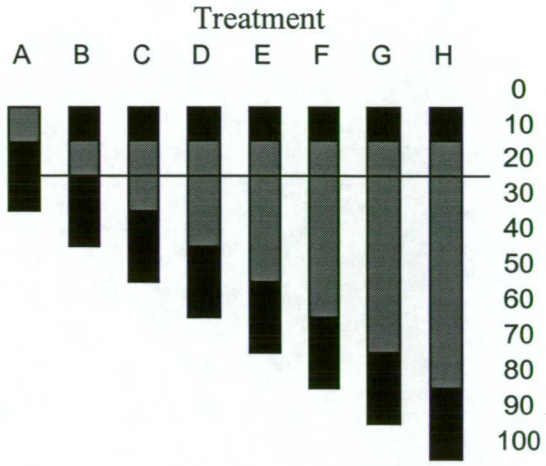

Figure 4.1. Depth of burial of billets and covering of areas of bark with sisalation paper (Depth of attack trial 4.5) (Sisalation paper indicated by hatch and exposed bark by black)

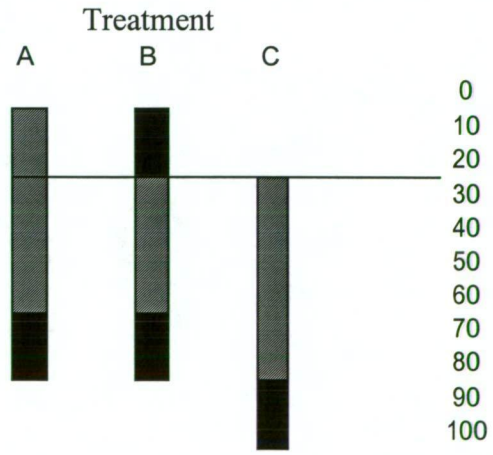

Figure 4.2. Depth of burial of billets and covering of areas of bark with sisalation paper (Depth of attack trial 4.6) (Sisalation paper indicated by hatch and exposed bark by black)

4.3.4. End-coating Experiment

Eighteen billets 60 cm long, were cut from a uniform *P. radiata* tree. Three billets were buried vertically 40 cm in the soil. The remaining fifteen billets were coated with mastic on each end and any patches where the bark had been removed. Once the mastic was dry, the billets were stored at room temperature until required. At monthly intervals three billets were taken out into the field and buried as above, with the moisture content of the bark being recorded. Each month the previously buried billets were lifted to check for *H. ater* invasion and then reburied. Invasion was noted by the presence of beetles, galleries and frass production.

4.3.5. Soaking Experiment

4.3.5.1. Soaking experiment introductory trial 4.7

Eight 60 cm long billets were cut from two freshly felled 17 year old *P. radiata* trees. These are referred to as the 'fresh' billets. The average moisture content of billets from both trees were 67.7% and 63.7% respectively.

In addition, at the same site, two dry trees which had been felled for approximately eight months, were cut into six 60 cm billets. These are referred to as 'dry' billets, with moisture contents of 30.5% and 54.9% respectively.

Three 'dry' billets and two 'fresh' billets were re-wetted by soaking in water for a period of seven days and three 'dry' billets and two 'fresh' billets were re-wetted by soaking in water for a period of 14 days during which time the moisture content rose. The soaking treatment involved the billets being completely submerged under water. The remaining four 'fresh' billets were endcoated with mastic with two of the billets stored for seven days and the remaining two billets stored for 14 days. The moisture content was also measured following the treatment period. At the establishment of the seven day and 14 day treatment two 'fresh' billets (Y and Z respectively) were harvested and buried with each series to act as controls. The time periods for the various treatments was chosen to correspond with weekly visits to the study site. For this

introductory trial extra billets from another experiment were utilised, corresponding to the unequal numbers of billets for some treatments. The billets were buried vertically 40 cm in the soil, and monitored after one, six and 27 weeks for invasion by *H. ater*. The number of entry sites were recorded at each visit, and at 27 weeks the number of adult beetles were also recorded.

4.3.5.2. Soaking experiment trial 4.8

In response to the change in slash management to one of slash retention, the aim of the soaking trials was to determine if dry unattractive host material had the potential to become attractive and suitable for *H. ater* following a rise in moisture content. This situation may arise in the field after rainfall. Fresh and dry billets were soaked in water for one, two, and four weeks by completely submerging under water. Then established simultaneously in the field along with billets which were not soaked and control billets as explained in the previous introductory trial. The following treatments were involved:

A	'Dry' 40 cm control billet
B	'Fresh' 40 cm control billet
E	'Dry' 40 cm billet soaked in water for 4 weeks
D	'Dry' 40 cm billet soaked in water for 2 weeks
C	'Dry' 40 cm billet soaked in water for 1 week
H	'Fresh' 40 cm billet soaked in water for 4 weeks
G	'Fresh' 40 cm billet soaked in water for 2 weeks
F	'Fresh' 40 cm billet soaked in water for 1 week
K	'Fresh' 40 cm billet endcoated for 4 weeks
J	'Fresh' 40 cm billet endcoated for 2 weeks
I	'Fresh' 40 cm billet endcoated for 1 week

Each treatment was replicated six times at the one site, with each series of eleven billets arranged in a row and separated by approximately six metres from the next replicate series. The billets were buried vertically with approximately 26 cm of the billet below the soil surface. Although similar sized billets were selected, the billets were standardised by measuring the bark surface area of each billet. After the establishment of the billets in the soil, they were lifted and

reburied at weekly intervals for a period of ten weeks to monitor for *H. ater* invasion with the number of entry holes recorded at each inspection.

'Dry' billets were obtained from *P. radiata* trees which had been felled more than 18 months, and 'fresh' billets were obtained from *P. radiata* trees which were felled just prior to when they were required.

The soaked billets were fully submerged in a small dam for the stated duration of one, two and four weeks. The fresh endcoated billets had both ends of each billet sealed with mastic following harvest and were stored in a shed in the field away from contact with the soil and general field conditions. The 'fresh' and 'dry' control billets were harvested on the day of trial establishment.

When the billets were harvested and again before trial establishment, samples of bark and wood were taken from each billet at three sites, for moisture content analysis.

4.3.5.3. Soaking experiment trial 4.9

The same procedure as described for trial 4.8 was followed except the following treatments were involved in trial 4.9:

O	'Dry' 40 cm control billet
P	'Fresh' 40 cm control billet
L	'Dry' 40 cm billet soaked in water for 8 weeks
C	'Dry' 40 cm billet soaked in water for 1 week
M	'Fresh' 40 cm billet soaked in water for 8 weeks
F	'Fresh' 40 cm billet soaked in water for 1 week
N	'Fresh' 40 cm billet endcoated for 8 weeks
I	'Fresh' 40 cm billet endcoated for 1 week

Each treatment was replicated six times at the one site, with each series of eight billets arranged in a row, separated by approximately six metres from the next replicate series. The billets were buried vertically with approximately 26 cm of the billet below the soil surface.

After the establishment of the billets in the soil, they were lifted and reburied at weekly intervals for a period of ten weeks to monitor for *H. ater* invasion with the number of entry holes recorded each week.

4.3.5.4. Soaking experiment trial 4.10

The same procedure as described for trial 4.8 was followed except the following treatments were involved in trial 4.10:

A	'Dry' 40 cm control billet
B	'Fresh' 40 cm control billet
F	'Dry' 40 cm billet soaked in water for 6 weeks
E	'Dry' 40 cm billet soaked in water for 4 weeks
D	'Dry' 40 cm billet soaked in water for 2 weeks
C	'Dry' 40 cm billet soaked in water for 1 week
J	'Fresh' 40 cm billet soaked in water for 6 weeks
I	'Fresh' 40 cm billet soaked in water for 4 weeks
H	'Fresh' 40 cm billet soaked in water for 2 weeks
G	'Fresh' 40 cm billet soaked in water for 1 week
N	'Fresh' 40 cm billet endcoated for 6 weeks
M	'Fresh' 40 cm billet endcoated for 4 weeks
L	'Fresh' 40 cm billet endcoated for 2 weeks
K	'Fresh' 40 cm billet endcoated for 1 week

Each treatment was replicated six times at the one site, with each series of 14 billets arranged in a row, separated by approximately six metres from the next replicate series. The billets were buried vertically with approximately 26 cm of the billet below the soil surface.

At weekly intervals the billets were lifted to monitor for *H. ater* invasion with the number of entry holes recorded and then reburied for a period of six weeks. At the completion of the trial, the bark was removed from each billet and the number of adult beetles present recorded.

4.3.6. Sustained and Extended Soaking Experiment

Three 30 cm long control billets were buried with 10 cm of the billet above the soil surface and three 30 cm long billets were buried with restricted drainage, being buried in a bucket to prevent normal water drainage. The billets were lifted, examined and then replaced on a monthly basis for a period of three months, and then the bark was removed from the billets and the number of adult beetles present recorded.

4.3.7. Stump Suitability

Different harvested coupes with stumps of six months, one, two and three years of age were investigated to determine how long stumps remain suitable for *H. ater* brood development and the number of stumps which are attacked by *H. ater*. At least 80 stumps of each age category were investigated being selected randomly over each coupe. Stumps were dissected to a depth of approximately 30 cm below the soil surface, with the bark being removed and any *H. ater* life stages present or evidence of previous occupation recorded.

BEETLE ASPECTS

4.3.8. Sound Production

4.3.8.1. Recording of beetle sound

In the laboratory, a small condenser microphone (9 mm diameter) was inserted into a glass vial (15 mm diameter) containing 20 adult male *H. ater* beetles and positioned 1 mm above the beetles. The vial was then inserted into a lead cannister (25 mm diameter) and enclosed in acoustic foam to prevent ambient sound being recorded. Using a high quality Grass instrument AM7 audio medical grade amplifier it was possible to record the sound emitted by the beetles.

4.3.8.2. Analysis of beetle sound

The recorded beetle sound was examined to determine the frequency using Cool Edit (digital sound editing Windows program).

4.3.8.3. Stridulatory structures

The undersurfaces of the elytra of both male and female adult *H. ater* were examined with the aid of scanning electron microscopy (SEM) for sound producing or stridulatory structures.

4.4. RESULTS

HOST ASPECTS

4.4.1. Substrates Attacked

Survey results summarised in Table 4.1 indicate that the only slash items attacked by *H. ater* were those that had been in contact with the soil either by lying on the soil surface or partially within the soil. No attack was recorded in any slash held above the soil surface. Therefore *H. ater* does not attack slash which lacks the soil-bark interface.

For all slash treated areas the majority of the slash held within the soil was attacked by the black pine bark beetle.

		Location of slash					
		Above soil		On soil surface		Within soil	
Treatment	n (slash items)	% total	% attacked	% total	% attacked	% total	% attacked
Slash Retained	569	56.5	0	35.9	47	7.6	97
Slash Cleared	276	24.0	0	66.3	89	9.7	98
Slash (Slasher)	286	7.6	0	80.4	9	12.0	58
Slash (Spot cultivator)	364	58.1	0	37.2	4	4.7	88

Table 4.1. Results of the various slash treatments showing the position of the slash in relation to the soil and attack by *H. ater*

4.4.2. Bark Thickness

Adult *H. ater* beetles exhibit a strong attraction to *P. radiata* billets moving into buried billets cut from mature (~17 year old) trees within seven days of establishment. Large numbers of adult beetles may share unit host material as shown in Tables 4.2, 4.3 and 4.4.

The thickness of *P. radiata* bark was found to influence the number of adult *H. ater* beetles that were attracted and ultimately supported per unit area of bark. There was a direct relationship between bark thickness and the number of

beetles invading the bark, as shown in Figure 4.3. This relationship was shown to be statistically significant with a p-value of 0.0001 (Appendix 5).

In trials 4.2 and 4.3 the majority of the population consisted of larval stages with some pupae present after 12 weeks when the billets were lifted as shown in Table 4.3 and 4.4. Immature *H. ater* stages were able to successfully complete their development within the range of bark thicknesses of two to 24 mm. Table 4.3 shows that *H. ater* is capable of developing within thin bark to a minimum of two mm thick. Being that *H. ater* pupae and adults measure less than two mm in width, this enables development within thin bark if appropriate moisture content is maintained.

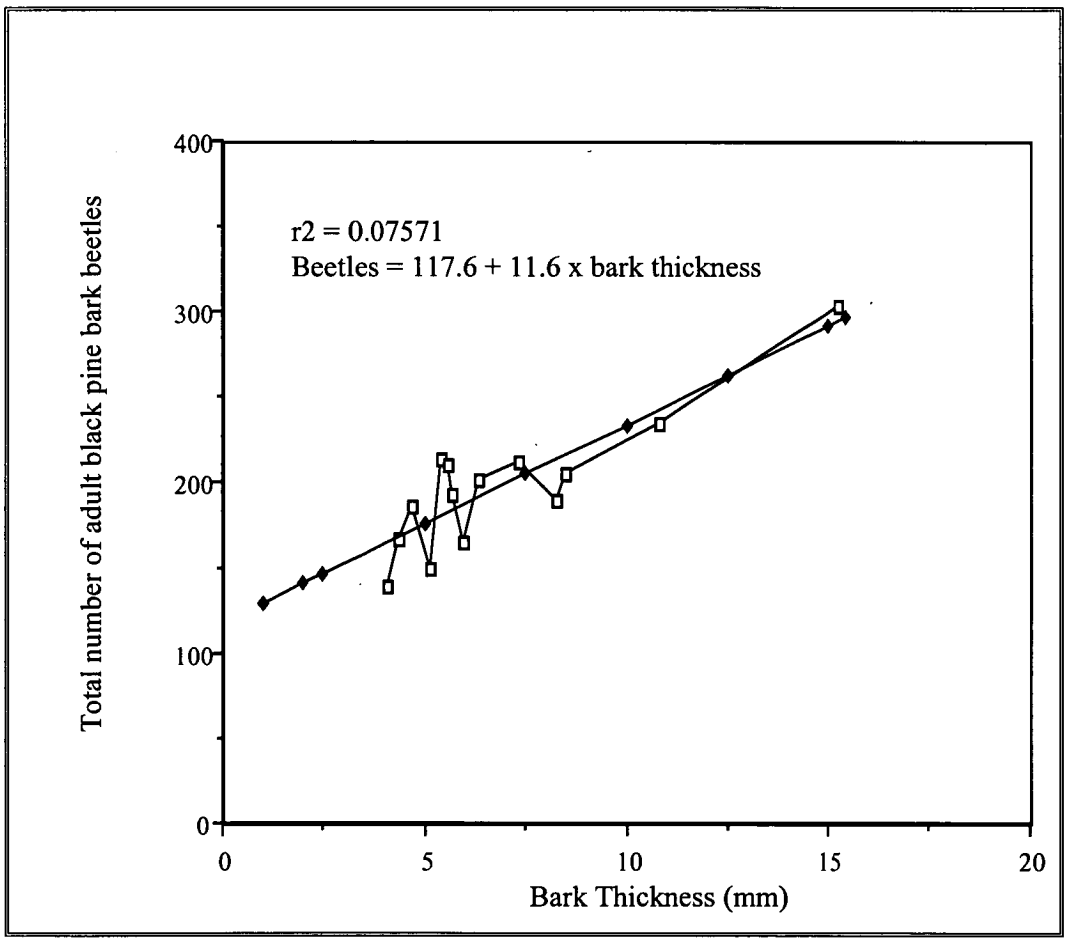

Figure 4.3. Number of adult *H. ater* beetles attracted to billets of different bark thicknesses (Bark thickness trial 4.1)

0.6 m Billets from base	Diameter (mm)	Bark & Phloem Thickness (mm)	Total Number Adults	Number Adults Per m^2
1	333.0	15.25	303	483
2	284.0	10.80	235	439
3	251.5	8.50	205	432
4	245.5	8.25	189	408
5	231.0	7.35	212	487
6	217.0	6.35	202	494
7	209.5	5.95	166	420
8	195.0	5.70	193	525
9	182.0	5.55	211	615
10	171.5	5.40	213	659
11	161.5	5.10	150	493
12	154.5	4.70	187	642
13	141.5	4.35	168	630
14	115.0	4.10	139	641

Table 4.2. Number of adult *H. ater* beetles associated with billets of various bark thicknesses eight weeks after establishment (Bark thickness trial 4.1)

0.6 m Billets from base	Diameter (mm)	Bark & Phloem Thickness (mm)	Total Number Adults	Total Number Pupae	Total Number Larvae	Total Number Live Stages	Number Live Stages Per m^2
1	162.0	13.75	2	6	577	585	1916
2	150.0	10.45	0	18	135	153	541
3	142.0	6.83	0	12	50	62	232
4	133.3	4.53	0	5	303	308	1226
5	124.8	3.78	0	14	104	118	502
6	117.8	3.25	0	35	50	85	383
7	119.7	3.10	0	5	47	52	230
8	115.4	2.98	1	10	50	61	280
9	101.5	2.88	0	3	304	307	1605
10	94.9	2.85	0	0	17	17	95
11	86.8	2.83	0	0	107	107	654
12	77.5	2.70	2	3	91	96	657
13	70.9	2.60	1	5	56	62	464
14	64.0	2.48	0	0	23	23	191
15	56.9	2.28	0	0	10	10	93
16	48.4	2.08	0	0	160	160	1754

Table 4.3. Number of *H. ater* life stages associated with billets of various bark thicknesses 12 weeks after establishment (Bark thickness trial 4.2)

0.6 m Billets from base	Diameter (mm)	Bark & Phloem Thickness (mm)	Total Number Larvae & Pupae	Total Number Adults	Total Number Live Stages	Number Live Stages Per m^2
1	333	24.0	41	9	50	80
2	333	23.5	152	16	168	270
3	305	20.0	139	6	145	252
4	290	19.0	22	14	36	66
5	285	14.5	46	8	54	101
6	280	13.5	3	4	7	13
7	280	13.0	37	2	39	74
8	240	12.0	49	14	63	139
9	245	10.5	144	3	147	318
10	220	9.0	93	4	97	234
11	215	9.5	30	12	42	108
12	190	9.5	26	3	29	81
13	190	9.5	247	33	280	782
14	180	8.0	7	0	7	21
15	165	6.0	53	1	54	174
16	165	5.5	239	0	239	768
17	140	5.0	22	0	22	83
18	130	5.5	65	0	65	267
19	125	5.5	4	0	4	17
20	105	4.8	6	2	8	40
21	90	4.3	15	1	16	94

Table 4.4. Number of *H. ater* life stages associated with billets of various bark thicknesses 12 weeks after establishment (Bark thickness trial 4.3)

4.4.3. Depth of Attack

4.4.3.1. Depth of attack trial 4.4

When billets were examined 12 weeks after establishment the insect population had nearly completed a generation. Numbers of individual galleries were recorded, as an indicator of the intensity of attack as shown in Table 4.5.

All billets that were completely buried to both 20 cm and 40 cm below the soil surface showed no sign of invasion. All billets vertically buried but either exposed at the soil surface or protruding 20 cm above the soil surface were occupied by *H. ater* but only in that section of the billet in direct contact with the soil. The number of galleries were higher in the billets which protruded above the soil surface compared to the billets that were buried with just the top cross section exposed at the soil surface.

Burial depth to bottom of billet	Mean number galleries ± SE
40 cm	35.2 ± 10.5
60 cm	14.6 ± 4.6
80 cm	0
100 cm	0

Table 4.5. *H. ater* attack of 60 cm vertical billets buried to different depths

4.4.3.2. Depth of attack trial 4.5

The extent of *H. ater* attack prevented the counting of individual galleries, so the site of attack on each billet was recorded. All control billets were attacked extensively from soil level to the bottom of each billet with numerous galleries and production of frass.

All treated billets were attacked only below the aluminium sisalation paper cover with no attack either above or behind the sisalation paper cover (except D and F which were not attacked). In billets E and G there was some attack behind the sisalation paper but only for a small distance from the bottom having invaded through the exposed end.

4.4.3.3. Depth of attack trial 4.6

The control billets were invaded by *H. ater* from the soil level to the bottom of each billet with galleries being indistinguishable due to the abundance of frass. Both treatments A and B, showed *H. ater* invasion at the bottom of each billet with some galleries extending underneath the aluminium sisalation paper. The number of galleries and adult beetles present were the same for both treatments. One billet of treatment C (billet not exposed and protected by sisalation paper) was not invaded while the other billet exhibited the presence of *H. ater* only at the base.

4.4.4. End-coating Experiment

For the first two months all of the billets were attacked following their burial, with the fresh November billets being attacked within three days of

establishment. The bark moisture content decreased each month as shown in Table 4.6, with the January billets having a moisture content of 51% and only two out of the three billets being attractive to *H. ater*. The February and March series having a moisture content of 48% and 45% respectively were unattractive to *H. ater* for several months, until May when a small invasion was noted. This was due to significant rains that presumably rewetted the host material. With subsequent billet inspections all of the billets showed *H. ater* invasion.

Month	Moisture content (%)
November	68.1
December	60.8
January	51.0
February	48.0
March	44.9
May	49.7

Table 4.6. Bark moisture content of end-coated billets over time

4.4.5. Soaking Experiment

4.4.5.1. Soaking experiment introductory trial 4.7

The 'dry' billets which were soaked for both seven and 14 days were found to be attractive and attacked by *H. ater* within three days from establishment. The 'dry' billets had initial moisture contents of 30.5% and 54.9% respectively, which rose to 47.0% and 73.8% following treatment. All of the treated billets were attacked within the first week of establishment as shown in Table 4.7. As shown in Figure 4.4, after six weeks the 'fresh' control billets and those soaked for 14 days were the most invaded. The attractiveness of 'fresh' and 'dry' billets were enhanced by the re-wetting treatment, as the longer soaking period of 14 days resulted in more entry sites for both 'fresh' and 'dry' billets compared to the seven days soaking period.

Billet Treatment	Average Number of Entry Sites			Adults
	1 week	6 weeks	27 weeks	27 weeks
'fresh' soaked 7 days	3.0	24.0	75.0	28.0
'fresh' endcoated 7 days	1.0	25.0	39.0	14.0
'fresh' control Y	5.0	43.0	55.0	23.0
'dry' soaked 7 days	3.3	18.0	33.7	12.3
'fresh' soaked 14 days	7.0	38.5	97.5	42.5
'fresh' endcoated 14 days	1.0	18.0	65.0	10.0
'fresh' control Z	4.0	30.0	60.0	8.0
'dry' soaked 14 days	4.5	25.0	58.5	24.5

Table 4.7. *H. ater* attack of soaking experiment introductory trial billets

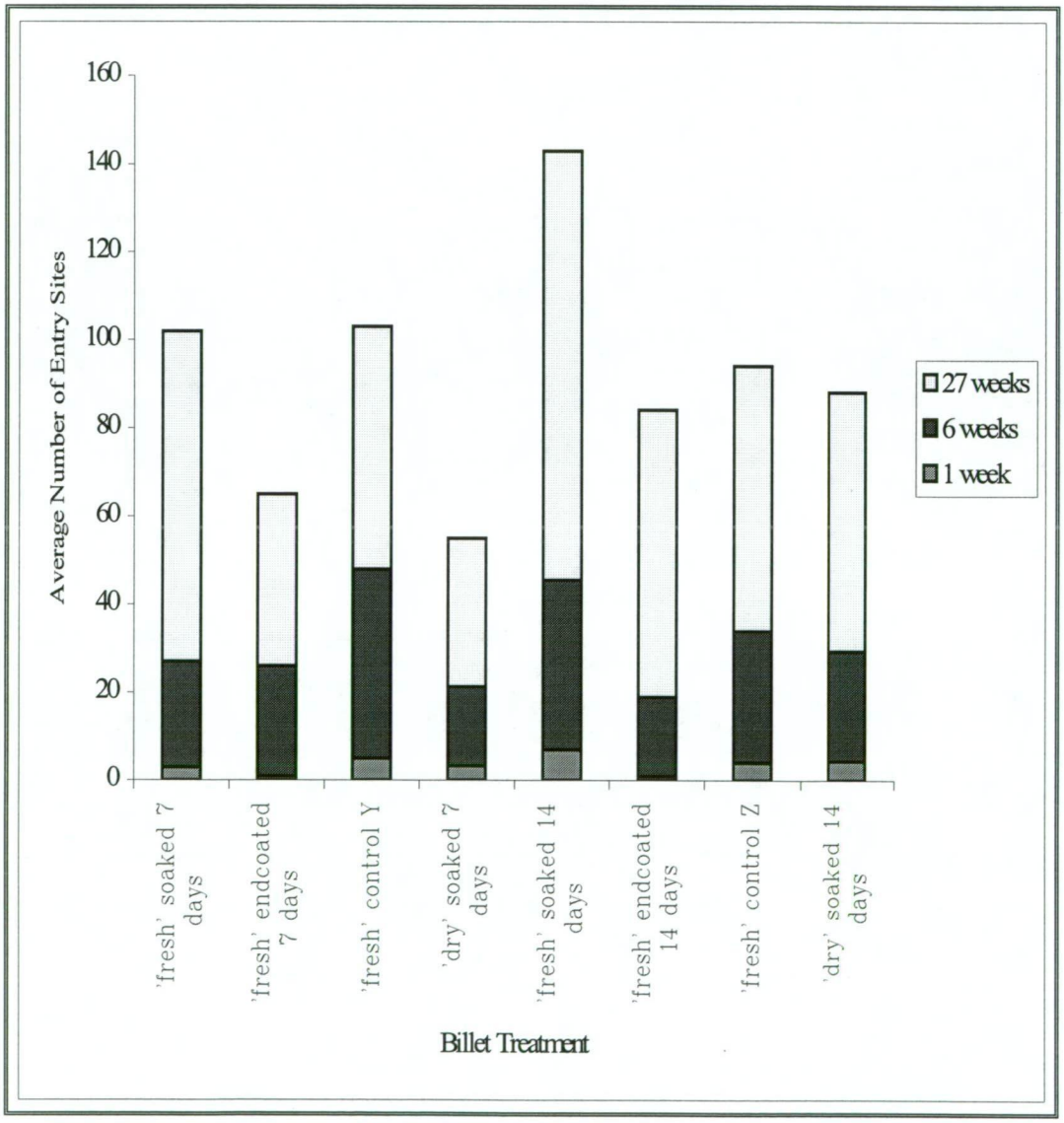

Figure 4.4. Average number of entry sites over time of soaking experiment introductory trial billets

4.4.5.2. Soaking experiment trials 4.8, 4.9 and 4.10

The average results for the three soaking experiment trials are shown diagrammatically in Figures 4.5, 4.6 and 4.7 respectively. The same general trends are evident over the three trials with the soaking treatment enhancing the 'attractiveness' of both 'fresh' and 'dry' billets. The duration of soaking appears to influence the number of beetles attracted evident in the number of entry holes, with the longer soaking duration resulting in a more 'attractive' billet. Even though there appeared to be trends evident in the results, they were not shown to be statistically significant when the treatments were compared on the number of entry holes per cm^2 and per cm^3 of *P. radiata* for soaking experiment trials 4.8 and 4.9 (Appendix 6 and 7). Soaking experiment trial 4.10 was shown to be marginally significant when the treatments were compared on the number of entry holes per cm^2 and per cm^3 of *P. radiata* with p-values of 0.001 and 0.003 respectively, but not statistically significant when the treatments were compared on the number of adult beetles per cm^2 and per cm^3 of *P. radiata* (Appendix 8).

The moisture content measurements taken from each billet before and after the treatments were applied are shown in Figures 4.8, 4.9 and 4.10 for soaking experiment trials 4.8, 4.9 and 4.10 respectively. Overall the moisture content of both the wood and bark of the 'dry' and 'fresh' billets rose following the soaking treatment. The rise in moisture content was more marked in the bark than the wood. The moisture content was maintained as a result of the endcoating treatment.

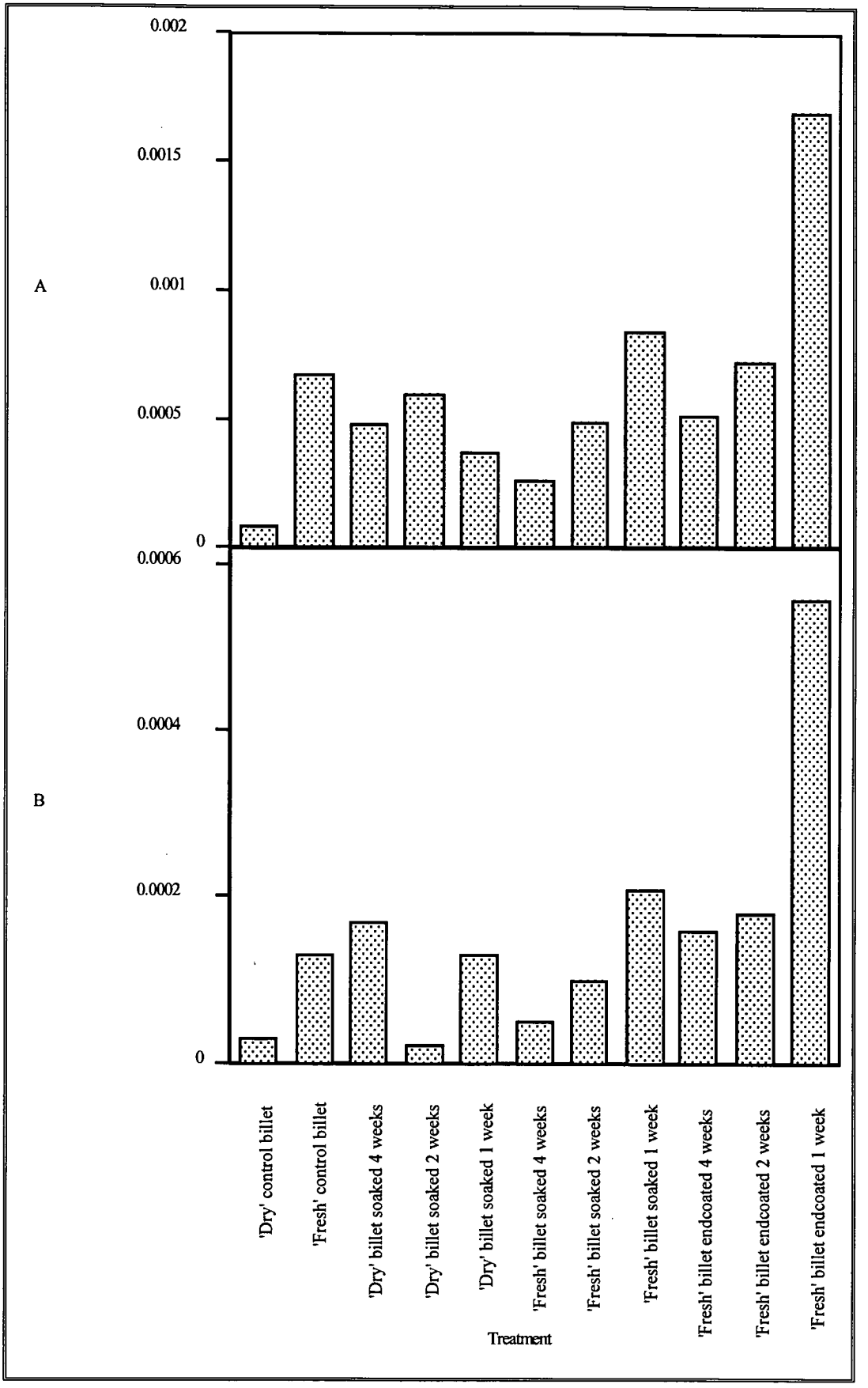

Figure 4.5. Average number of *H. ater* entry holes made in fully submerged water soaked billets A) per cm^2 and B) per cm^3 of *P. radiata* bark (Soaking experiment trial 4.8) (Appendix 6)

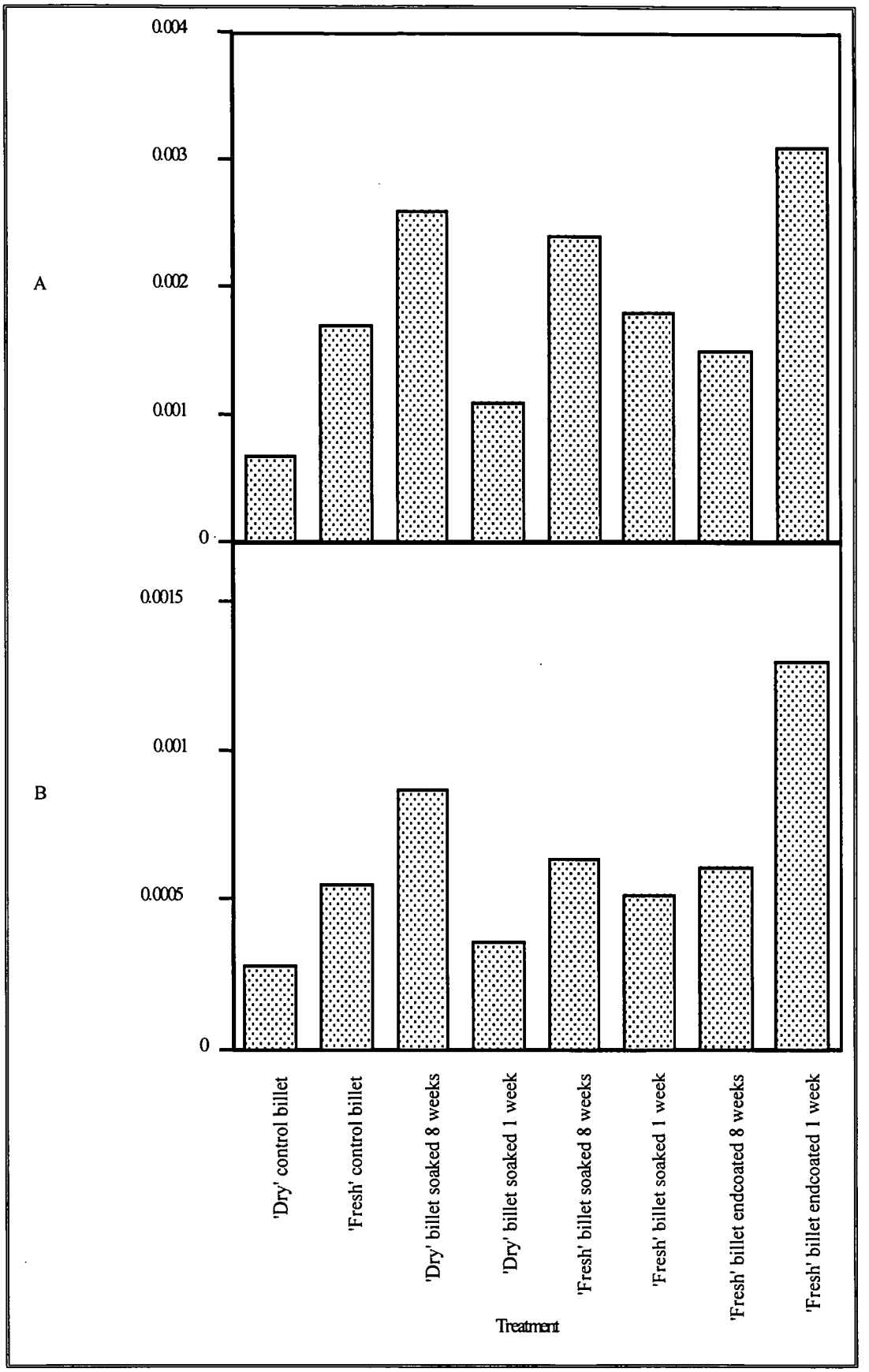

Figure 4.6. Average number of *H. ater* entry holes made in fully submerged water soaked billets A) per cm^2 and B) per cm^3 of *P. radiata* bark (Soaking experiment trial 4.9) (Appendix 7)

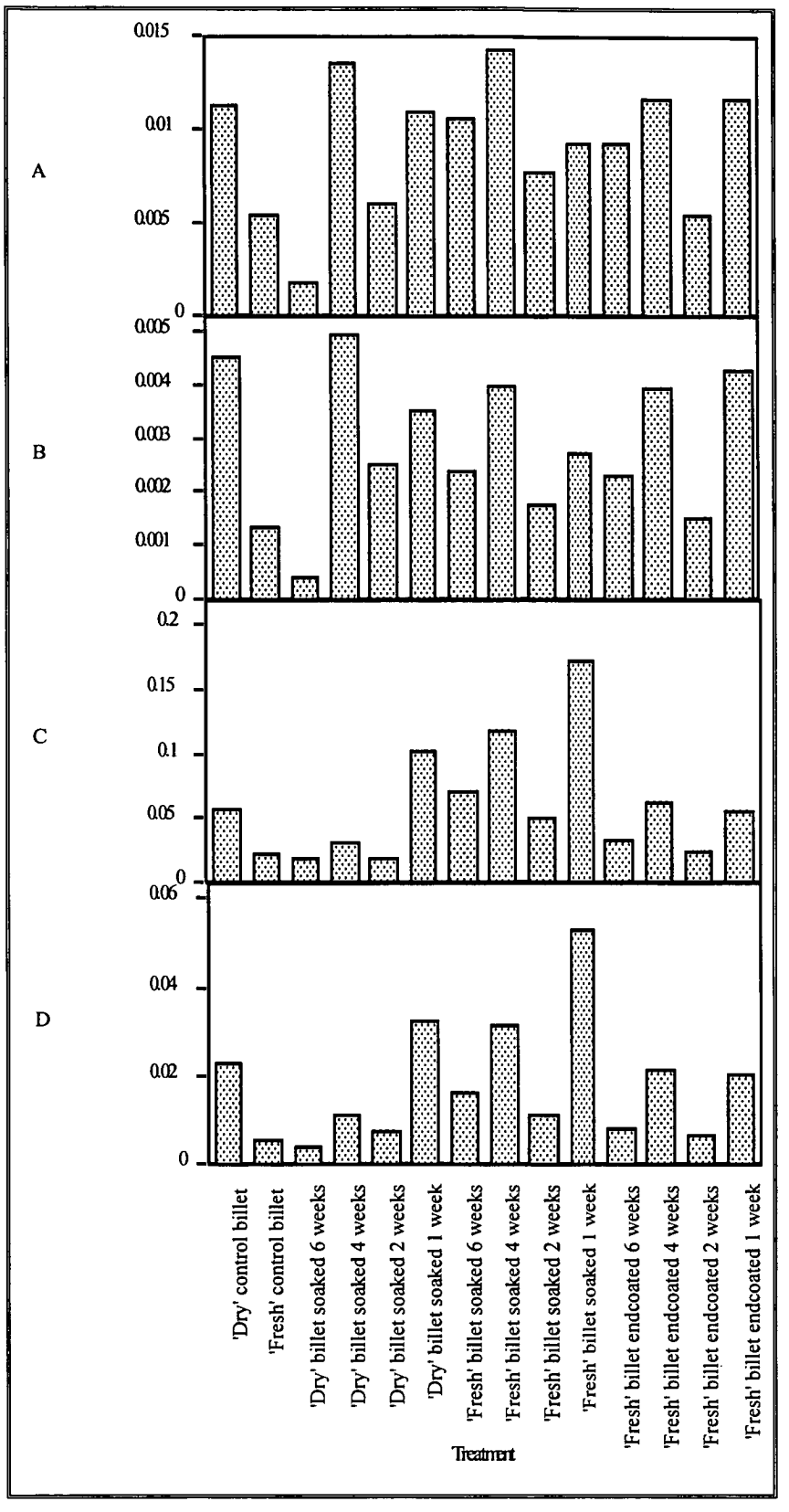

Figure 4.7. Average number of *H. ater* entry holes made and adult beetles present in fully submerged water soaked billets A) entry holes per cm^2 and B) per cm^3 of *P. radiata* bark C) adult beetles per cm^2 and D) per cm^3 of *P. radiata* bark (Soaking experiment trial 4.10) (Appendix 8)

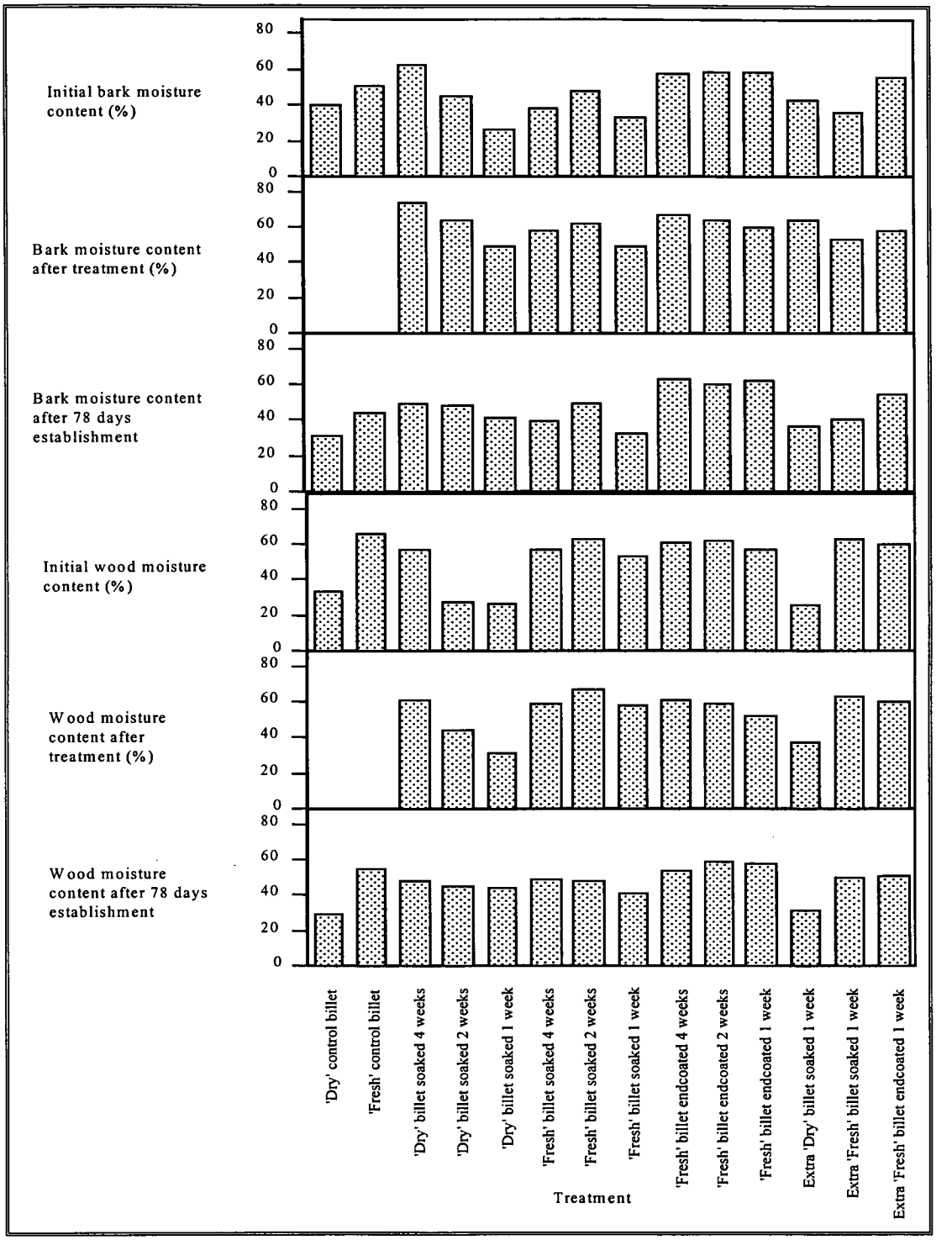

Figure 4.8. Moisture content of bark and wood of soaked billets before and after treatment and after establishment (Soaking experiment trial 4.8) (Appendix 6)

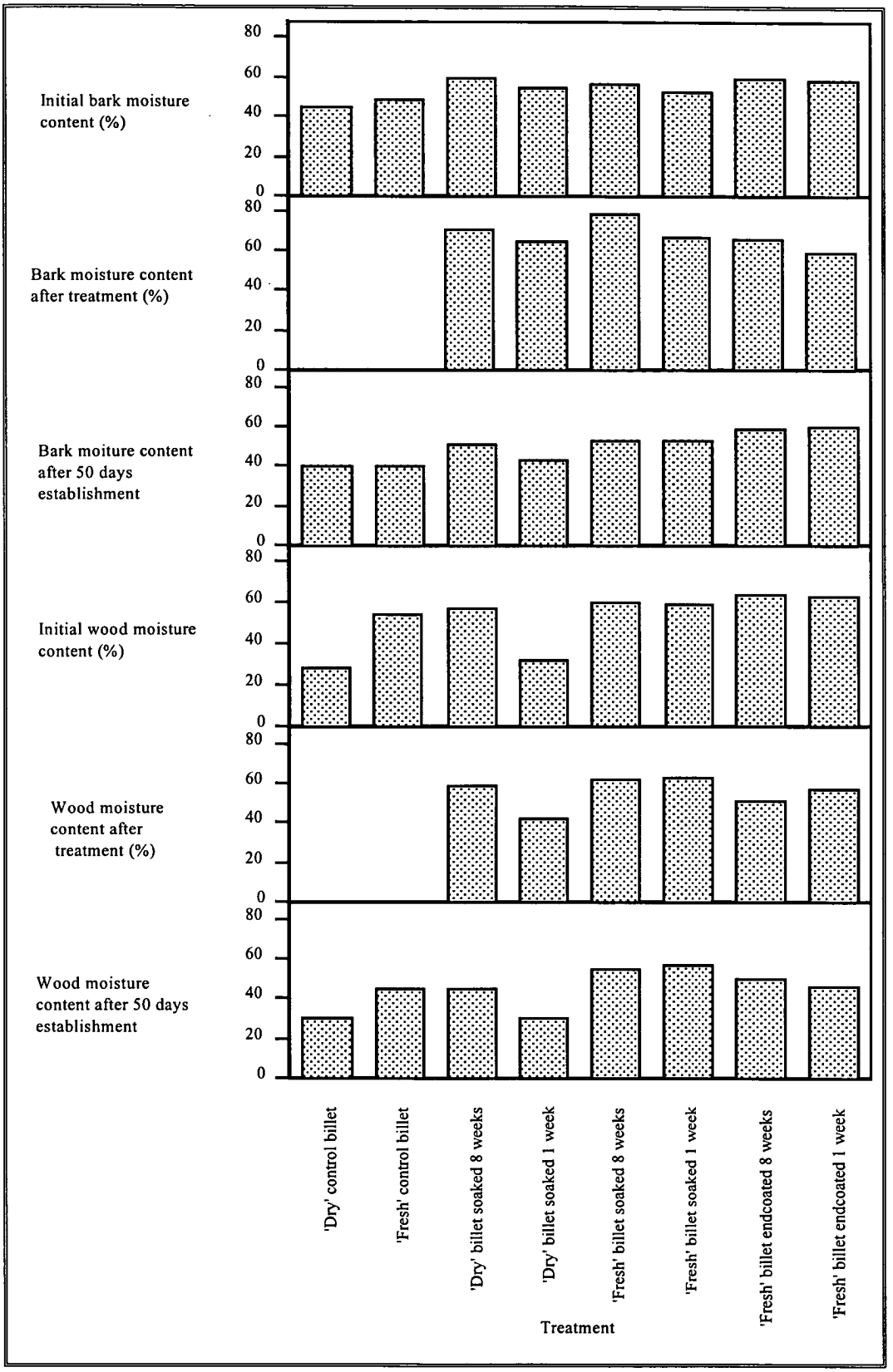

Figure 4.9. Moisture content of bark and wood of soaked billets before and after treatment and after establishment (Soaking experiment trial 4.9) (Appendix 7)

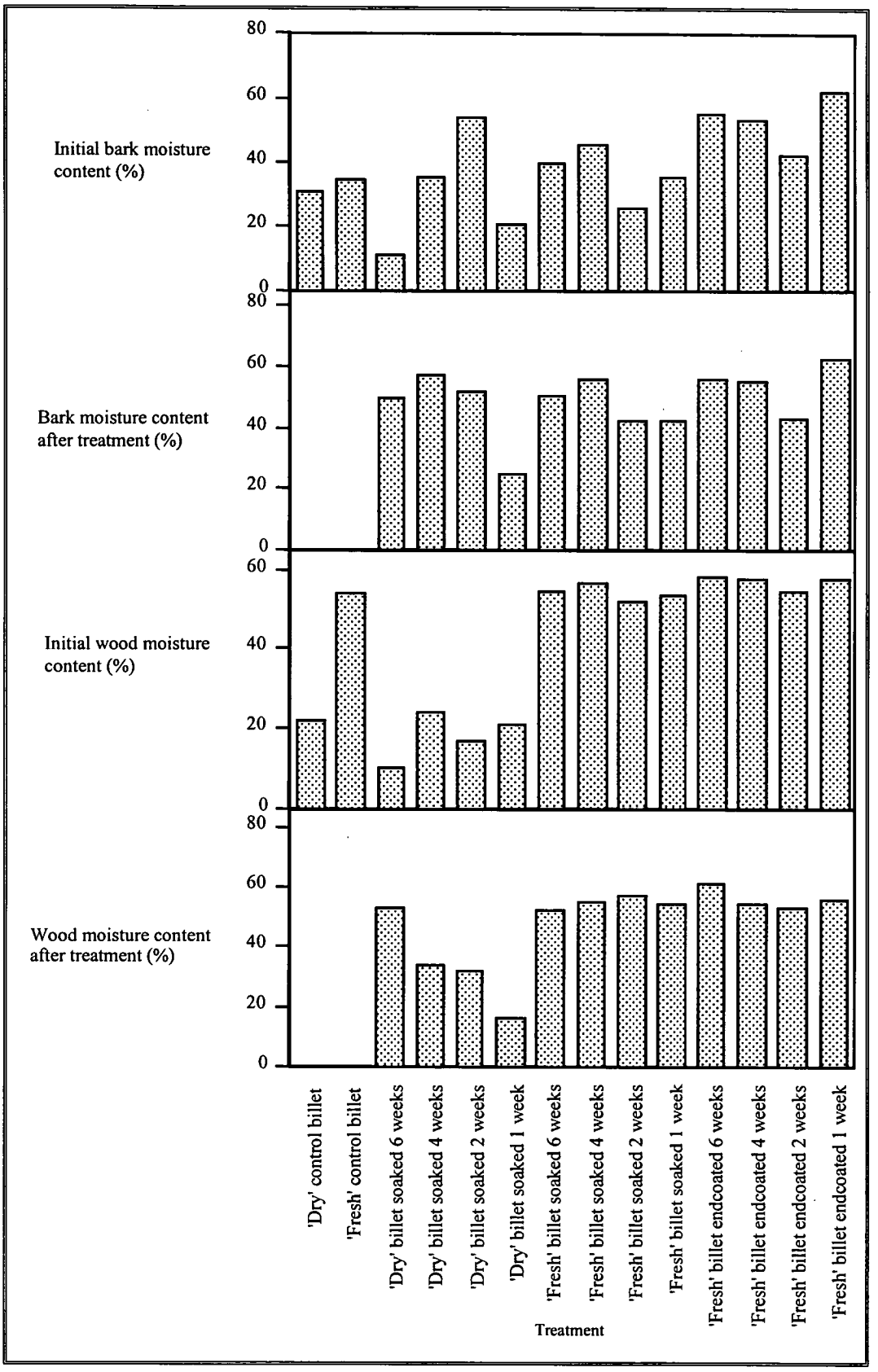

Figure 4.10. Moisture content of bark and wood of soaked billets before and after treatment (Soaking experiment trial 4.10) (Appendix 8)

4.4.6. Sustained and Extended Soaking Experiment

When the billets were examined one month after establishment all of the control billets were being heavily attacked, and only one of the restricted drainage billets had been slightly attacked. The soil in the buckets was waterlogged. Two months after establishment all of the control and drainage billets were being attacked. The number of adult beetles found in the billets after three months are shown in Table 4.8.

Treatment	Mean number adult beetles per $cm^2 \pm$ SE
Restricted Drainage	0.10 ± 0.08
Control	0.09 ± 0.02

Table 4.8. Number of adult *H. ater* beetles present in the control and restricted drainage billets

4.4.7. Stump Suitability

The results of the stump survey are summarised in Table 4.9. For all coupes investigated with stumps up to two years old 90% of all stumps were attacked by *H. ater*. Only 60% of stumps showed evidence of *H. ater* invasion in the coupe containing three year old stumps. This coupe was separated from other harvested areas by approximately one km being one of the first harvested sites, and the beetle population was not as high in this coupe corresponding to less available logging residue and hence *H. ater* breeding material. Stumps are capable of supporting *H. ater* development up to two years after harvesting with no live *H. ater* stages present in stumps three years old as shown in Figure 4.11.

Years since harvest	% Stumps attacked	% Stumps with live stages
0.5	89	71
1	89	63
2	91	38
3	60	0

Table 4.9. Stump survey showing the number of stumps attacked by *H. ater* and containing living life stages for each stump age category

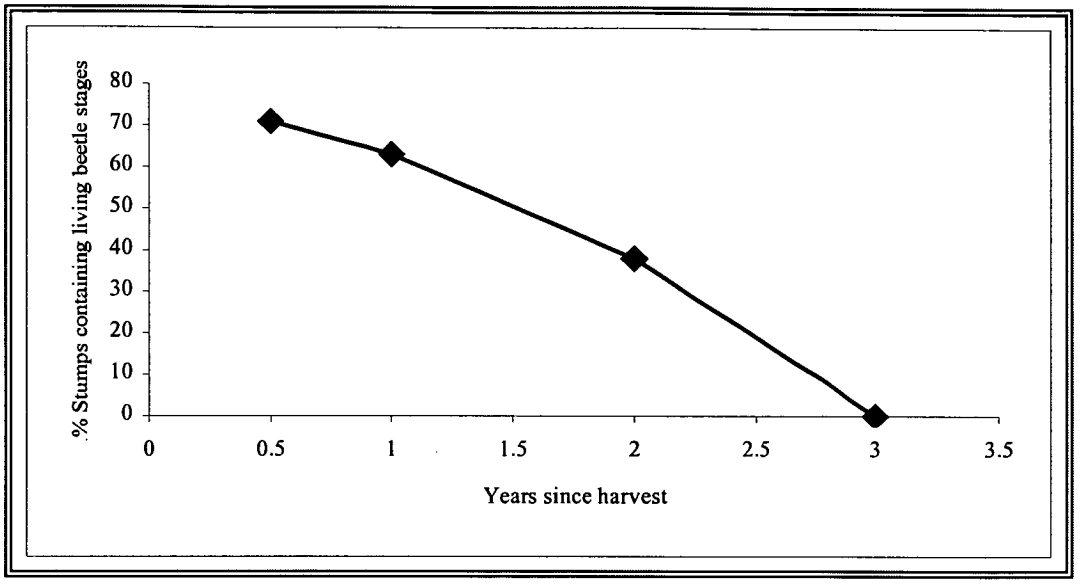

Figure 4.11. Number of stumps of various ages from harvesting containing living *H. ater* life stages

BEETLE ASPECTS

4.4.8. Sound Production

Male *H. ater* adult beetles possess the ability to produce sound or stridulate with a distinct chirping noise being audible when beetles are held close to the ear. No sound was detected from female *H. ater* adult beetles. SEM showed the presence of a series of parallel, transverse ridges on the undersurfaces of both the right and left elytra of male and female *H. ater* adult beetles. The structures were present at the apice of each elytron extending only a short distance along each side. The structures present on the elytra of male *H. ater* adult beetles are shown in Plate 4.1 and 4.2. The analysis of the recorded sound of one chirp from a male *H. ater* adult beetle is shown as a bitmap in Figure 4.12. The highest amplitude section of the chirp is shown in the frequency spectrogram of Figure 4.13. This shows that the loudest portion of the sound emitted by the beetles is between a frequency of 2000 to 8000 Hz.

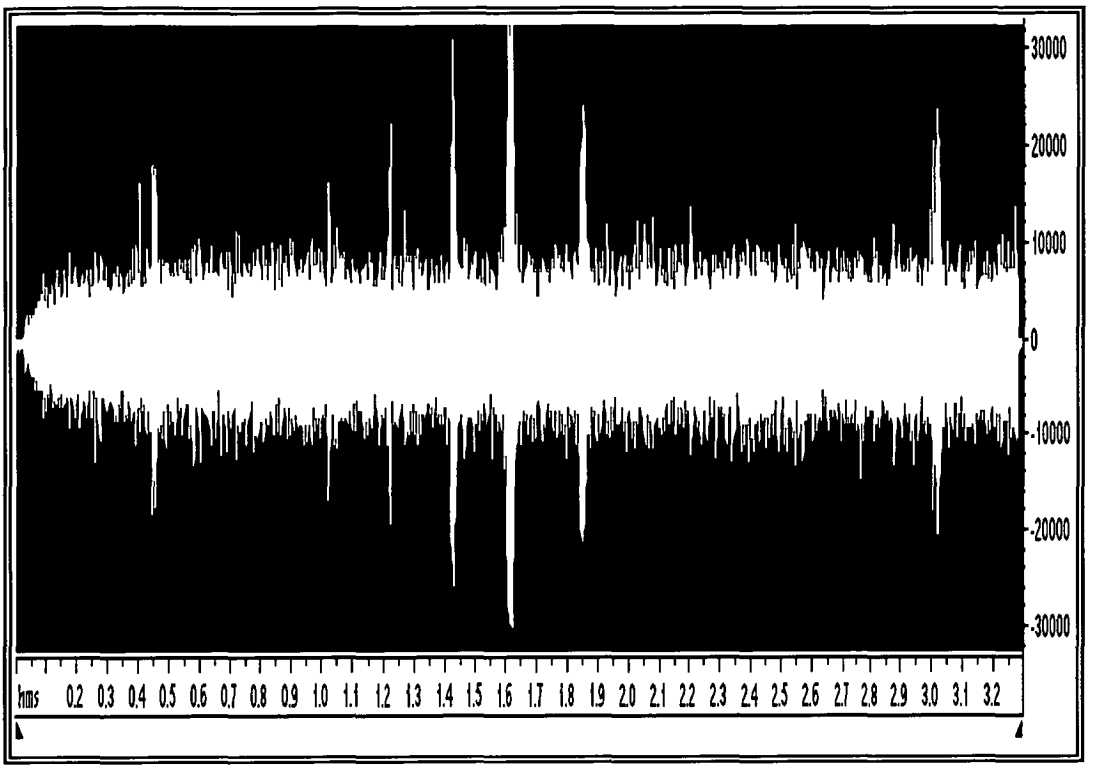

Figure 4.12. Bitmap of one chirp of male *H. ater* adult beetles

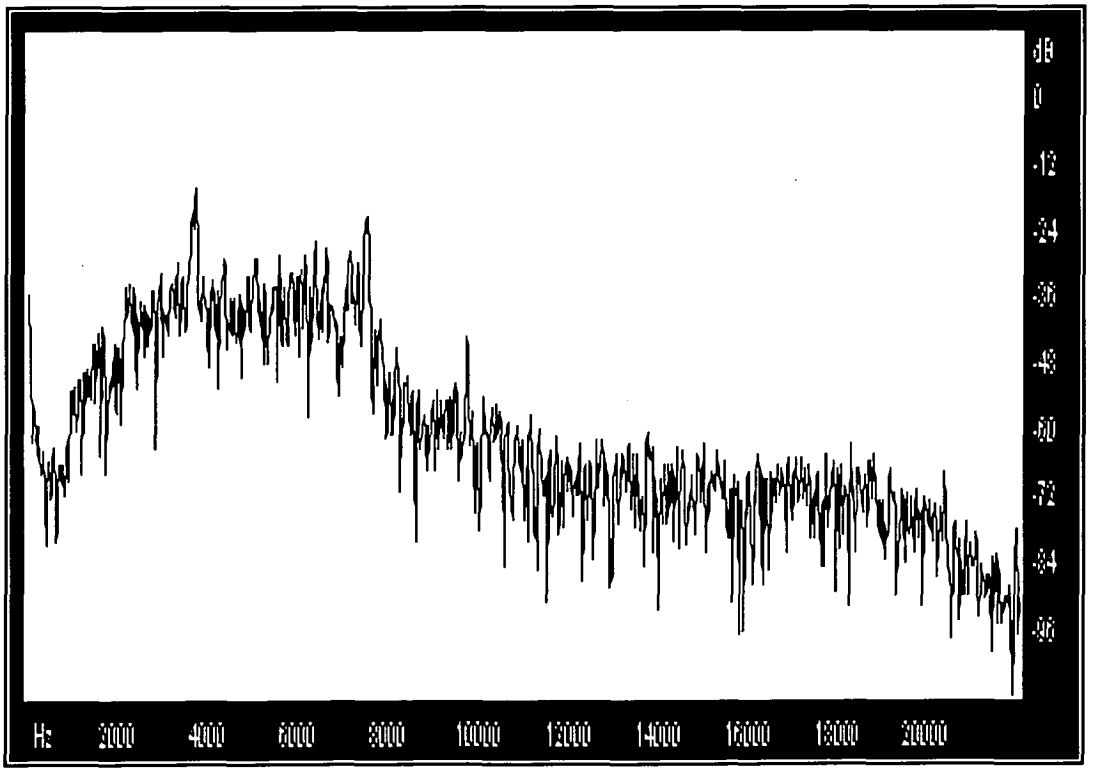

Figure 4.13. Frequency spectrogram of the centre part of the bitmap (loudest section)

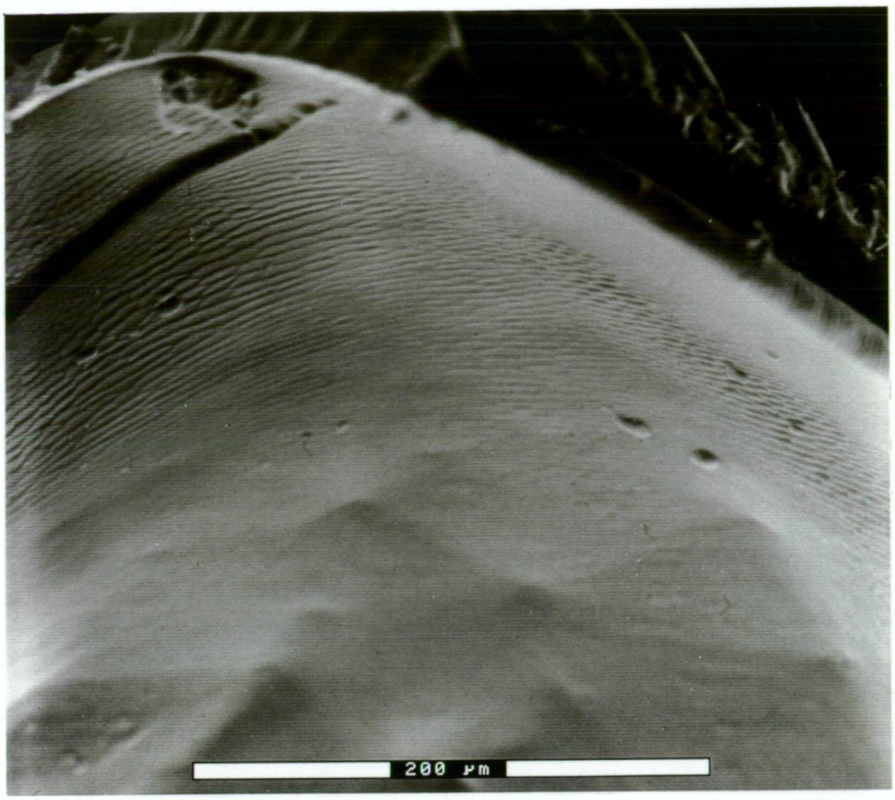

Plate 4.1. Undersurface elytron of male *H. ater* adult beetle showing the stridulatory structure (x 250)

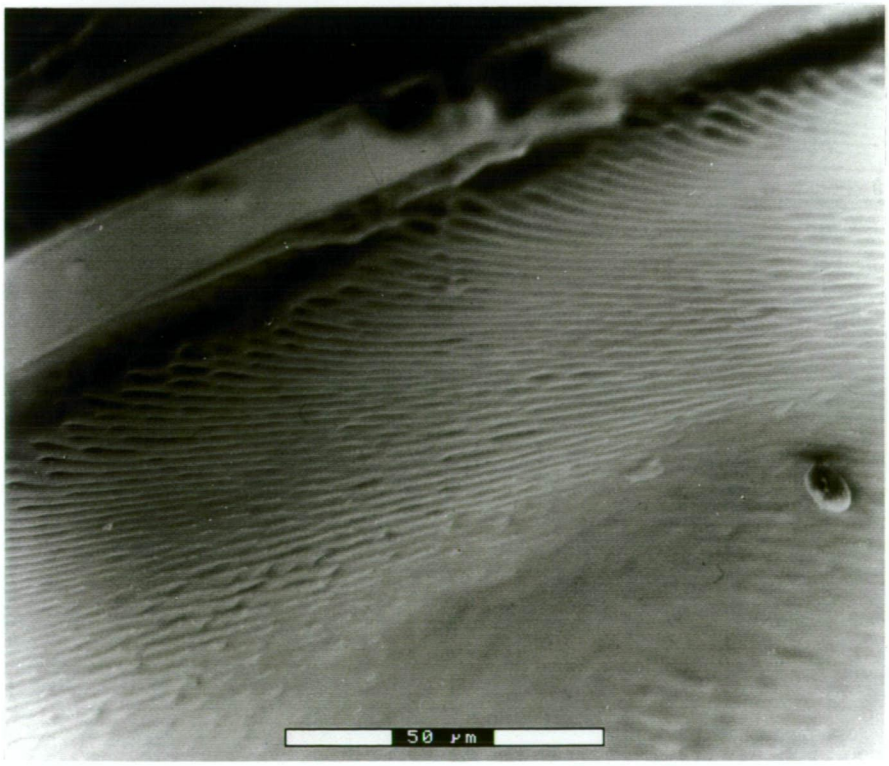

Plate 4.2. Stridulatory structure or 'pars stridens' present on the elytron of male *H. ater* adult beetles (x 600)

4.5. DISCUSSION

HOST ASPECTS

4.5.1. Substrates Attacked

Sites from which slash had been removed were very clean although the slash that remained was in direct contact with the soil and the majority of moderately large pieces provided ideal breeding sites.

Those sites, in which the slash had been pushed to either side to allow for the establishment of a planting row, were characterised by the majority of the slash being suspended above the soil surface. The same situation was found for the slash in those sites which had been left 'as is' with the exception of where approximately one metre wide holes had been made by a spot cultivator at intervals to allow the planting of seedlings.

Sites which were treated by chipping the slash could not sustain any *H. ater* invasion as the chips redistributed on to the soil surface had dried and had little intact bark. The chipping procedure was the most appropriate slash treatment to minimise potential *H. ater* breeding sites.

For all sites the majority of the slash that occurred either on the soil surface or partially within the soil had been attacked, except in those instances where slash was isolated from soil by a layer of pine needles. Bain (1977), Milligan (1978), Eldridge (1983) and Neumann (1987) noted that close proximity to the soil appeared to be a requirement for *Hylastes* attack, favouring the log surface in direct contact with the soil (Clark (1932), Zondag (1982)). It is the larger slash within the soil which is of major concern as it will maintain its moisture content and provide potential breeding sites whereas most slash on the soil surface will dry out before the brood has developed.

4.5.2. Bark Thickness

In an initial bark thickness investigation it was thought that a bark thickness greater than 10 mm was required to enable the complete development of *H. ater* (Griggs 1993). Following more detailed investigations it was confirmed that *H. ater* will feed in bark of any thickness and that they are able to successfully complete their entire life cycle within bark which is 2 mm wide. So, the number of potential breeding sites are much greater than originally thought as long as the moisture content is suitable, and there is direct contact with the soil. These results contrast with Swan (1942) who reported that breeding takes place in thick bark.

The slash with thinner bark acts mainly as a food source for beetles, but is capable of supporting a developing brood through to maturation if greater than 2 mm thick, if in direct contact with the soil and there is an absence of drying conditions such as occurs during the cooler months of the year.

4.5.3. Depth of Attack

In the initial investigation, (Depth of attack trial 4.4) it was found that billets completely buried below the soil surface were not attacked. While those billets buried just below the soil surface or with some of the billet protruding above the soil surface were invaded, with less invasion recorded in billets buried just below the soil surface. This indicates that harvesting stumps as close to the soil surface as possible would minimise invasion. Also, the complete burial of host material would prevent invasion.

The distance that adult beetles would go down below the soil surface in search of suitable host material was found to occur from the soil level down 10-80 cm below to the bottom of each billet. When billets were covered with sisalation paper, all billets were only attacked below the paper. Thus adult beetles had descended and made their entry only into the bark at the bottom of the billet. *H. ater* will attack *P. radiata* material to depths of 80 cm below the soil surface, as long as there is some of the billet protruding above the soil surface showing the

attraction was either due to a visual sign or odours released from the *P. radiata* material.

The third trial showed an equal attraction of beetles to billets when the billets were exposed or completely covered with sisalation paper to the soil surface. The presence of the sisalation paper did not affect beetle attraction, but did prevent the exploitation of those areas covered with sisalation paper, due to limited access.

4.5.4. End-coating Experiment

The endcoating experiment showed that moisture content appears to play an important role in the attraction and suitability of *P. radiata* for *H. ater*. This supports Swan's (1942) observation that pine bark is attractive at felling and for several months afterwards until at some stage it becomes unsuitable for *H. ater*. A moisture content around 50% 'appears' to be the borderline moisture content which is suitable for invasion by *H. ater*. The mastic treatment did not maintain the moisture content in the *P. radiata* billets for as long as originally thought. The results indicate that *P. radiata* can remain attractive up to three months following harvesting, but also that billets are able to become attractive and suitable for brood development following a rise in moisture content.

4.5.5. Soaking Experiment

The aim of the soaking experiments to see if re-wetting 'dry' billets could make them attractive again for *H. ater* was shown to be true. 'Dry' billets are the least attractive to *H. ater*, with the duration of soaking increasing the number of adult beetles that were attracted to the 'dry' billets making them as 'attractive' as 'fresh' billets. Soaking resulted in a rise in moisture content in both 'dry' and 'fresh' billets. The evaporative process followng a soaking treatment would assist the release of volatile host compounds contributing to *H. ater* invasion.

The ability of 'dry' *P. radiata* host material to become attractive again to *H. ater* and suitable for brood development is a serious problem in regard to the

forest management practice of slash conservation. *P. radiata* slash has the ability to remain attractive and suitable for *H. ater* development much longer than an initial invasion experienced following harvesting.

4.5.6. Sustained and Extended Soaking Experiment

The effect of the restricted drainage reduced initial *H. ater* attack. One month after establishment the billets and soil in the buckets were too wet, for significant *H. ater* invasion. Two months after establishment the restricted drainage billets showed an equivalent level of invasion to the control billets. This was due to drier weather conditions experienced during the second month.

The moisture content of the *P. radiata* material plays a role in its suitability for *H. ater* invasion and occupation, with very wet material being unsuitable for entry. This indicates that material exposed to waterlogging is unsuitable for *H. ater* invasion, but can be attacked following removal of such conditions.

4.5.7. Stump Suitability

The majority of stumps remaining after harvesting are utilised by *H. ater* with living life stages found in stumps six months to two years after harvesting, with *H. ater* having vacated stumps after three years. These results differ from that of Swan (1942) in South Australia, who found that bark more than one year old was unsuitable for *H. ater*.

The stumps provide a large host resource for brood development and the build up of large numbers of beetles particularly during the first year after harvesting. Stumps are able to provide suitable conditions longer than slash which is lying on top of the soil surface.

BEETLE ASPECTS

4.5.8. Sound Production

The ability by *H. ater* to produce sound or stridulate has only been documented by Wichmann (1912), Munro (1916), Marcu (1931) and Barr (1969).

The production of sound by only male *H. ater* adult beetles in this study agrees with Barr (1969) and Stark (1982) who describe the presence of stridulatory organs in the sex opposite to which initiates the entrance tunnel.

The stridulatory structure observed on the undersurfaces of both elytra in male and female *H. ater* adult beetles is the same as that described by Marcu (1931) comprising the 'pars stridens' of an elytra-abdominal tergite type. The presence of the stridulatory organ on the undersurface of the elytra in both sexes but the ability to produce sound possessed only by male beetles may be due to the presence of a 'plectrum' on the abdominal tergite in only male beetles. Munro (1916) describes the presence of a stridulatory structure on the seventh tergite of male *H. ater* beetles. The abdominal tergites were not examined.

Sound production in the Scolytidae is highly developed (Stark 1982) but its role has been an area of limited research. The production of sound by male *H. ater* beetles may assist in host colonisation once both sexes have arrived at suitable host material in communication with other beetles in mate pairing or defence.

CHAPTER 5

HOST SELECTION AND ATTRACTION - CHEMICAL ASPECTS

5.1. INTRODUCTION

The black pine bark beetle, *H. ater*, attacks its host substrate of *P. radiata* stumps, logs, and logging slash to feed and provide breeding sites. In their search for food and suitable oviposition sites adult *H. ater* beetles also feed on the bark of the entire root structure of *P. radiata* seedlings resulting in their death.

The selection by bark beetles of suitable hosts may either be the result of landing randomly on slash, stumps, logs or seedlings or a response to host produced attractants and/or visual cues, or a combination of the two mechanisms. Once a host is selected, the adult beetles may produce compounds which sometimes combine with host produced volatile compounds. The responses of adult beetles to host and insect produced attractants are termed primary and secondary host selection respectively (Stark 1982, Coulson & Witter 1984).

Chemical aspects of the host selection and attraction process of *H. ater* were investigated with the aim of determining what chemical compounds and mechanisms were involved in *H. ater* host recognition and colonisation process. There is a general lack of work on the chemical aspects of *Hylastes* spp. host selection and colonisation stages in particular for *H. ater*. Determination of the attraction process and the mechanisms involved would assist in the overall understanding of *H. ater* and its management. There is the potential to use compounds involved in host selection and attraction in monitoring or suppression.

From field observations it appeared that a few pioneer beetles would arrive at suitable host material and this event was then followed by a mass attack. The involvement of *P. radiata* host volatile compounds and/or insect produced volatile compounds needed to be determined as there is little work of this nature on *H. ater*.

The first aim was to isolate any volatiles occurring within the gut of male and female beetles at different stages of their development and to determine the

chemical identity of compounds that may act as sex attractants or repellents. The standard procedures utilised in scolytid semiochemical research were employed using whole beetle extraction and headspace techniques.

Secondly, a number of synthetic volatile compounds which have been reported as sex attractants or repellents with primary bark beetles were obtained from Phero Tech Inc., Vancouver BC (Canada) and trialed in the field in an attempt to determine if such compounds are involved in *H. ater* host selection and colonisation stages. The compounds, *cis*-verbenol (*cis*-2-pinen-4-ol), verbenone (2-pinen-4-one), *trans*-verbenol (*trans*-2-pinen-4-ol), α-pinene, sulcatol (6-methyl-5-hepten-2-ol), MCH (3-methyl-2-cyclohexen-1-one), and MCOL (1-methyl-2-cyclohexen-1-ol) obtained from Phero Tech Inc. (Canada) were trialed in combination with *P. radiata* billets. Lindgren multiple funnel traps also purchased from Phero Tech Inc. (Canada), were used to trial the three primary bark beetle pheromones ipsenol (2-methyl-6-methylene-7-octen-4-ol), ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) and frontalin (1,5-dimethyl-6,8-dioxabicyclo(3.2.1)octane). The multiple funnel traps are a very efficient method of trapping many bark beetles in the Northern Hemisphere in pheromone based research, survey and mass trapping of scolytid beetles (Lindgren 1983). Also various volatile solutions which resemble or are naturally occurring compounds in *P. radiata* or result from biological breakdown processes were trialed in some small field trials using simple bottle traps as well as *P. radiata* bark. The aim was to assist in understanding the role of *P. radiata* host compounds in host selection and attraction. Simple laboratory bioassays were also performed on all compounds trialed under field conditions.

Thirdly, samples of feeding beetles were analysed for a duration of several weeks with changes in volatile compounds monitored over time and compared to host material without beetles. The resulting frass was analysed and volatile compounds extracted via a soxhlet extraction.

Fourthly, *P. radiata* material of various physiological conditions were analysed to determine any changes in the presence of volatile compounds.

5.2. LITERATURE REVIEW

5.2.1. General

Prior to dispersal, a beetle must emerge from its host or overwintering site, in which it has matured or reemerge from an earlier dispersal (Neumann 1987). Before emergence diapause must be broken and the flight muscles mature (Borden 1982). Emergence and dispersal are influenced by environmental conditions particularly temperature (Stark 1982). As described by Borden (1982) "emergence may be temperature induced and follows a distinct diel periodicity, based on environmental conditions or endogenous circadian rhythms".

The two stages of host selection and colonisation involve the initial search for suitable hosts by 'pioneer' beetles, followed by colonisation or mass attack of the selected trees by large numbers of beetles (Berryman 1974). There are many factors involved in the orientation of bark beetles to their host trees (Perttunen 1957).

5.2.2. Host Selection

The life cycle of all bark beetles begins with an adult beetle locating a host (Coulson & Witter 1984). The initial discovery is termed primary host selection and may involve perception of the host by olfactory means in response to compounds released by host material; or may be a result of random searching or visual cues (Chapman 1967, Norris 1972, Stark 1982, Neumann 1987). The condition of the host can influence colonisation and hence subsequent brood development (Coulson & Witter 1984).

Once a host is selected, the adult beetles may produce compounds which sometimes combine with host produced volatile compounds, resulting in the attraction of large numbers of additional adults (Coulson & Witter 1984). The response of adult beetles to insect and host produced attractants is termed secondary host selection (Stark 1982, Coulson & Witter 1984, Neumann 1987).

5.2.3. Semiochemicals

Scolytid beetles utilise an intricate complex of chemical messengers, commonly known as semiochemicals ('semiochemical' meaning message delivering chemical), to communicate with each other and other species (Vite' 1971, Brand et al 1979, Borden 1982). Pheromones have been defined as semiochemicals which induce a behavioural or physiological response in members of the same species (Shorey 1977). Pheromones are chemical messengers produced by insects (Atkins 1968).

In the Scolytidae, the major pheromones are aggregation pheromones which may cause both sexes to aggregate on a host tree or log (Borden 1974, 1977). There are also other pheromones which act to regulate attack and population density, and these are known as epideictic or spacing pheromones, antiattractants, inhibitors or antiaggregation pheromones (Borden 1977, Stark 1982).

Numerous scolytid-produced semiochemicals and several host plant volatiles may also serve as interspecific messengers. In this case they are called allomones (Brown 1968, Borden 1977) when their action results in adaptive benefit to the emitting organism (e.g. defensive secretions) or kairomones (Brown et al 1970, Borden 1977) when their action results in adaptive benefit to the perceiving organism (e.g. substances that enable predators to locate their prey) (Brand et al 1979).

Vite' and Pitman (1967) define bark beetle attractants in terms of their origin and release mechanism, as specific host odours, insect-activated host attractants, and insect-produced attractants. Firstly, *Hylastes* spp. and *Hylurgops* spp. colonise their hosts in response to the release of odours, which may be associated with the physiological condition of the host. Secondly, other bark beetles (e.g. *Dendroctonus valens* LeConte) increase the release and/or the production of an aggregating stimulant through the insects physical activity of boring, resulting in a more rapid colonisation. Thirdly, insect produced volatiles or pheromones act as attractants (e.g. *Dendroctonus frontalis* Zimmermann).

5.2.4. Primary Attraction

Primary attraction refers only to attraction emanating from host trees or logs (Borden & Stokkink 1971). Odours from the host may be a consequence of a certain physiological condition, for example resins as exudates from cut conifers which results in the primary attraction. Many bark beetles exhibit clear primary attraction including *Dendroctonus pseudotsugae* Hopkins, *Dendroctonus rufipennis* Kirby, *S. multistriatus*, *Scolytus ventralis* LeConte and *Ips typographus* Linnaeus (Moeck et al 1981, Borden 1982).

Primary attraction was exhibited by the two bark beetles *H. gracilis* and *Ips latidens* LeConte to high-girdled lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann as a result of the volatiles released due to the wound response of the tree and then later due to the stressed phloem (Miller et al 1986).

When searching for suitable host substrate many scolytid beetles utilise host volatiles such as monoterpenes and the degradation product ethanol (Chapman 1967). Moeck (1970) described the attractiveness of ethanol for the ambrosia beetle, *Trypodendron lineatum* Olivier. Studies by Schroeder and Lindelow (1989) showed that ethanol attracted the scolytid beetles *Tomicus piniperda* L., *Hylurgops palliatus* Gyllenhal, *T. lineatum*, *Hylastes cunicularius* Erichson, *Hylastes brunneus* Erichson, *Anisandrus dispar* F. and *Hylastes opacus* Erichson. The monoterpene α-pinene attracted all species except for *T. lineatum*, *H. cunicularius* and *A. dispar*. The combined treatment of ethanol and α-pinene resulted in synergistically increased attraction of all species except for *H. opacus* and *A. dispar*. A combination of α-pinene and ethanol was also shown by Bauer and Vite' (1975) to be attractive to *T. lineatum*.

Ethanol was shown by Moeck (1981) to act as a strong attractant for the spruce beetle, *D. rufipennis*, by inducing attack on the spruce tree, *Picea engelmannii* Parry. Ethanol-baited traps were more attractive than unbaited traps to scolytid beetles (Montgomery & Wargo 1983).

A method of detecting weevil activity described by Rieske and Raffa (1993) exploits the insects attraction to host volatiles. *Hylobius pales* Herbst,

Pachylobius picivorus Germar, and *Hylobius radicis* Buchanan can be successfully trapped using pitfall traps baited with ethanol and turpentine.

The concentration of monoterpenes is an important factor in determining bark beetle orientation (Perttunen 1957). It was noted by Perttunen (1957) that *H. ater* was able to tolerate high concentrations of α-pinene (120 mm^3), while the secondary species, *H. palliatus* exhibited a strong repellent effect. The difference in response can be correlated to the condition of the host material which each species selects (Perttunen 1957). It was also shown that *H. ater* exhibited a weak but significant attraction to a lower concentration of α-pinene (12 mm^3) (Perttunen 1957). (No units of α-pinene concentration were given, but it is assumed 120 mm^3 and 12 mm^3 referred to mm^3 of mercury).

The monoterpenes and related resinous compounds secreted by pine trees play an important role in ensuring their protection against many insects which would otherwise be successful in attacking them (Perttunen 1957). The resistance of healthy trees against bark beetle attack may be due at least in part to the secretion of these compounds. Although if a pine is damaged and weakened, or killed by harvesting, bark beetles may select it as their host. In such a pine, the concentration of monoterpenes must continually diminish, many chemical reactions occur in the phloem and new chemical substances are formed through oxidation and fermentation (Perttunen 1957).

Bark beetles respond to the release of compounds resulting from deterioration of the host tree often due to the weakening action of plant pathogens (Kamm & Buttery 1984). The bark beetle, *H. nigrinus*, colonises Douglas Fir infected with black-stain root disease caused by the fungal agent *Ceratocystis wageneri* Goheen and Cobb (Witcosky et al 1987). Experiments conducted by Witcosky et al (1987) "suggest either that infection leads to an increase in release of host attractants or that it results in the production by host, pathogen, or both of a new compound or compounds which enhance the attraction of *H. nigrinus*, and that olfactory cues released from injured or infected roots could contribute to the host selection process".

5.2.5. Secondary Attraction

Secondary attraction includes additional attraction which follows initial attack of a new host by 'pioneer' beetles. It usually results in the aggregation and regulation of a natural population and mass attack of the host (Borden 1982). Secondary attraction may be an increase in release of primary attractants by the boring activity of the resident beetles, or the activity of the insect results in further attraction through the production of aggregating pheromones. Most often, however, it has proved to be a result of insect produced, attractive compounds (pheromones) which may act alone or in combination with host volatiles (Stark 1982).

Insect produced attractants are found in 20 bark beetle species belonging to the genera *Ips*, *Orthotomicus*, *Pityogenes*, *Trypodendron* and *Dendroctonus* (Hopping 1963). Borden and Stokkink (1971) detail the presence of pheromones in the same genera as well as *Hylesinus*, *Pseudohylesinus*, *Tomicus*, *Phloeosinus*, *Carphoborus*, *Polygraphus*, *Scolytus*, *Pityokteines*, *Cryphalus*, *Pityophthorus* and *Gnathotrichus*.

The semiochemicals forming part of their chemical ecology have been identified for many scolytid beetles.

For the Douglas-fir beetle, *D. pseudotsugae*, the aggregation pheromones have been identified as frontalin, seudenol (3-methylcyclohex-2-en-1-ol) and MCOL, and an antiaggregation pheromone identified as MCH (Rudinsky et al 1972, Lindgren et al 1992). The natural function of MCH is to reduce intraspecific competition by terminating attraction after a generally sufficient attack density has been achieved to overcome a tree's defences (Furniss et al 1981, 1982). Although uncertain as to how it actually acts it may either mask the attractive pheromones or repel beetles (Furniss et al 1982).

The pine engraver, *Ips pini* Say, attacks pine and spruce in North America (Teale et al 1991). Male beetles enter the host tree and release a chemical attractant which results in the colonisation of the host by responding male and female beetles (Teale et al 1991). The pheromone components of *I. pini* have

been found by numerous workers to consist of a combination of ipsdienol, lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one), (E)-myrcenol, and β-phellandrene (Miller & Borden 1990, Teale et al 1991).

The pheromone components of *Ips paraconfusus* Lanier comprise (-) ipsenol, (+)-*cis*-verbenol, and ipsdienol (Silverstein et al 1966).

Ipsenol acts as a pheromone for the bark beetle, *I. latidens* which attacks the lodgepole and ponderosa pines, *P. contorta* var. *latifolia* and *P. ponderosa* (Miller et al 1991).

The pheromone components of *I. typographus* include ipsdienol, ipsenol, methylbutenol (2-methyl-3-buten-2-ol) and *cis*-verbenol (Bakke et al 1977, Bakke 1985, Schlyter et al 1992).

The attractive pheromones of *S. multistriatus* have been identified as 4-methyl-3-heptanol, α-multistriatin, (2-4-dimethyl-5-ethyl-6,8-dioxabicyclo(3.2.1)octane) and α-cubebene (Peacock 1973, Cuthbert & Peacock 1979).

The pheromone components of the Southern pine beetle, *D. frontalis* have been found to consist of frontalin, *trans*-verbenol, verbenone, *endo*-brevicomin and myrtenold (Kinzer et al 1969, Coster 1970). Verbenone produced by male *D. frontalis* beetles inhibits male and female aggregation and overcolonisation of individual hosts (Rudinsky 1973).

Borden and McLean (1979) detail results that show that pheromone-based secondary attraction occurs in the ambrosia beetles, *Gnathotrichus retusus* LeConte and *Gnathotrichus sulcatus* LeConte.

The aggregation pheromones lineatin and sulcatol have been identified from *G. sulcatus* and *T. lineatum* (Borden et al 1976, Brand et al 1979) and for *T. lineatum* the host kairomones α-pinene and ethanol (Bakke 1983, Setter & Borden 1993).

The aggregation pheromones associated with the western pine beetle, *D. brevicomis* include *exo*-brevicomin, frontalin and myrcene, and the antiaggregation pheromones verbenone and ipsdienol (Bertram & Paine 1994).

Pitman and Vite' (1969) suggest that host and insect produced volatiles are utilised in the orientation of the mountain pine beetle, *D. ponderosae*. The semiochemicals myrcene, *trans*-verbenol, and *exo*-brevicomin have been identified as attractive for *D. ponderosae* attacking *P. contorta* var. *latifolia* and *P. ponderosa*, and verbenone as an antiaggregation pheromone (Borden et al 1983a, Conn et al 1983).

Another area of study in scolytids has been the chiral-specific activity of pheromones and the intra- and interspecific roles of their enantiomers (Brand et al 1979, Lindgren et al 1992). In understanding the chemical ecology of scolytids the ratio of production of the enantiomers and the response to the enantiomers alone and in combination with other attractants play an important role (Lindgren et al 1992).

5.2.6. Use of Semiochemicals in Management and Control

Vite' (1971) was one of the first to suggest the use of semiochemicals in pest management of primary bark beetles, in particular the southern pine bark beetle. An understanding of the chemical ecology of scolytids has enabled the use of semiochemicals in the research and control of many bark beetles via mass trapping, suppression, monitoring and containment (Mitchell 1981). The availability of synthetic pheromones has enabled investigations of bark beetle behaviour in both the laboratory and under field conditions (Brand et al 1979). Practical applications and potential use of pheromones in an integrated pest management system include trapping for monitoring, surveying and population suppression; luring to areas treated with pesticides or pathogens; and disruption of communication (Brand et al 1979).

Management programs for the Douglas-fir beetle, *D. pseudotsugae* include the use of the antiaggregation pheromone MCH integrated with other silvicultural and pheromone based strategies (Ross & Daterman 1995). Controlled-release formulations of the antiaggregation pheromone MCH were shown to be successful in reducing *D. pseudotsugae* attacks (Furniss et al 1974, 1977). Furniss et al

(1981) summarise the results of further investigations as "the application of a granular controlled-release formulation of the antiaggregation pheromone MCH applied prior to beetle flight by helicopter, reduced Douglas-fir beetle attacks by 92 to 97% and progeny by 93 to 99%". MCH applied by helicopter at a rate of 4.48 kg/ha to uninfested stands reduced attack by *D. pseudotsugae* by 96.4% compared to untreated areas (McGregor et al 1984). The attack density of Douglas-fir beetle on *P. menziesii* treated with MCH bubble caps was reduced to 3-7% of that on untreated trees (Lindgren et al 1988). MCH applied in bubble cap formulations at rates of 45-76 g/ha prevented Douglas-fir beetle infestation of live trees (Ross & Daterman 1995). Regulation of the amount and distribution of tree mortality by *D. pseudotsugae* was demonstrated with the use of aggregation pheromone baited traps (Ross & Daterman 1997). The combined use of the antiaggregation pheromone MCH around the perimeter and aggregation pheromone baited traps outside the boundary, was shown to be effective in reducing Douglas-fir beetle attack in small high value stands (Ross & Daterman 1994).

Richerson et al (1980) details the successful containment of the Southern pine beetle, *D. frontalis* within active infestations using the semiochemical Frontalure (a 1:2 mixture of frontalin and α-pinene).

Borden et al (1983b) details the successful use of semiochemical baiting applied as post logging treatments for the mountain pine beetle, *D. ponderosae.*

Gray and Borden (1989) detail work which shows the successful containment and concentration of mountain pine beetle, *D. ponderosae* infestations of lodgepole pine, *P. contorta* var. *latifolia*, by the use of the semiochemicals, *trans*-verbenol, *exo*-brevicomin and myrcene. Application of the antiaggregation pheromone, verbenone to lodgepole pine significantly reduced attack by *D. ponderosae*, compared to untreated areas (Lindgren et al 1989a, Shea et al 1992, Shore et al 1992).

The use of verbenone around the active front of an infestation to prevent continued expansion is approaching operational status (Clark et al 1996, Salom et

al 1997). The successful development of this method has been reported (Payne & Billings 1989, Salom et al 1992, Payne et al 1992, Billings et al 1995).

Pheromone baited traps form part of the overall integrated pest management program for *I. typographus* in Norway (Bakke 1989, 1991).

Reduced attack by *D. rufipennis* on *P. engelmannii* was demonstrated with the use of MCH bubble caps applied to susceptible trees (Lindgren et al 1989b).

The effective use of suppression traps baited with sulcatol for the ambrosia beetle, *G. sulcatus*, in a commercial sawmill was demonstrated by McLean and Borden (1979).

Richmond (1985) details trials of two non-insecticidal repellents synthetically produced from mixtures of terpene hydrocarbons which show promise as a preventive spray against attack of lodgepole pine by the primary pest, the mountain pine beetle, *D. ponderosae*.

5.3. MATERIALS AND METHODS

5.3.1. Insect Volatile Extraction

Fresh *P. radiata* billets established in the field were used as the source of adult beetles for experimentation. The adult stages were beetles excised from bark prior to emergence and naturally emerged beetles, as well as beetles at various intervals from emergence through feeding and establishment. Entry holes were marked each day for a period of time, to enable the collection of beetles of known feeding duration. Following collection, all beetles were handled identically and processed within a time frame of a few hours.

The method employed was that of Madden et al (1988) which was adapted from Butterfield (1983). Individual live beetles were placed in numbered vials on dry ice. The frozen beetles were then sexed, weighed and placed in 100 µl pentane in chilled 2 ml vials. The frozen sections were allowed to thaw and then 2 µl of 2-octanol solution was added to each vial. The sections were frozen again, and crushed with the flat head of a stainless-steel probe, and 2 µl of 3-octanol

solution added. The vials were allowed to thaw at room temperature, then shaken to ensure solvent penetration, then the tissue was refrozen and the pentane extract transferred to clean vials sealed with Teflon-lined caps, and stored at -20°C.

The solutions of 2- and 3-octanol were utilised as standards and prepared by adding 2.5 µl of each alcohol to 10 ml of pentane. The difference in peak height between these two materials was a general measure of evaporative losses in the extraction process.

All samples were analysed at the Central Science Laboratory (CSL), University of Tasmania by combined Gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard (HP) 5890 Gas Chromatograph coupled to a HP 5970B mass selective detector. Splitless 1 µl injections were made onto a HP-1 column (25 m x 0.32 mm, 0.52 µm film thickness). The injector temperature was 200°C, detector temperature 290°C and the GC oven programmed to operate from 30 to 290°C increasing at 10°C/min after an initial hold at 40°C for 1 min. Electron impact mass spectra were acquired at 70 eV, scanning from *m/z* 35 to 240 at a rate of 1.7 cycles per sec.

The levels of volatiles present within adult beetles were low, consisting primarily of terpenes. As a result selected ion monitoring (SIM) with the GC-MS analysis was also performed. Using this method target compounds were separated into three 'time windows'. Group one consisted of monoterpenes and 2- and 3-octanol (2-9 min), group two consisted of monoterpenes (9-12 min), and group three sesquiterpenes (12-22 min). Due to the low levels of volatiles detected from individual beetles, some samples were also prepared by pooling four to 10 *H. ater* beetles together. Beetles within each pooled group were of the same sex, and experience having been collected from the same *P. radiata* source.

A headspace volatile collection technique was also utilised by taking 2 ml of air from closed vials in which live *H. ater* beetles had been placed for one hour. The beetles were collected from the field one day prior to testing, with 10 beetles (five female and five male) of similar experience placed in each of four 2 ml vials.

Analysis of the headspace samples utilised the same procedure as previously described for analysis of whole beetle extracts. The only exception being the slow splitless cryogenic injection of the sample onto the HP-1 column using a liquid nitrogen trap.

5.3.2. Assessment of Potential Volatile Compounds

5.3.2.1. Field trials

Due to a lack of information regarding the chemical compounds involved in the host selection and attraction process of secondary bark beetles, including *H. ater*, a number of commercially available synthetic compounds were trialed with the aim of determining their involvement.

Five field trials were established in the same second rotation site at different times over a six month period from January to June 1996, using commercial bubble cap sachets of compounds in combination with *P. radiata* billets. The volatile compounds *cis*-verbenol, *trans*-verbenol, verbenone, sulcatol, MCOL, MCH, ipsenol, ispsdienol, and frontalin were obtained from Phero Tech Inc., Vancouver BC (Canada) in the form of bubble cap sachets. The α-pinene treatment was prepared by placing the α-pinene in 15 ml screw-cap polyethylene bottles. These compounds were selected as they are commercially available and economically priced.

For field trial 5.1, 28 billets 30 cm in length were cut from a freshly harvested *P. radiata* tree. All of the billets were kept together in order with each replicate consisting of seven billets. Each replicate of seven billets were buried vertically in a row with 1/3 above the soil surface with approximately one metre between each billet. The four replicates of seven billets were distinctly labelled and buried individually at random over the same general area. In each replicate of seven billets, three of the billets were controls and the remaining four billets had a volatile compound sachet attached on the southern side to the billet with string. The southern side was chosen to minimise contact with direct sun. The treatments were labelled A to E (A: *cis*-verbenol; B: verbenone; C: control; D: *trans*-

verbenol; E: α-pinene). All treatments were in a random order within each of the replicates while the controls maintained the same position on each end and in the middle of each row of each replicate, as shown in Figure 5.1. All billets were examined and the number of entry holes recorded every seven days for a period of three weeks. At the completion of the trial the bark was removed from each billet and numbers of *H. ater* and *H. ligniperda* beetles collected, counted and sexed.

As a result of this first trial, it was found that the number of entry holes needed to be recorded more often than every seven days, in order to effectively show trends. This was conducted with subsequent trials. The period of establishment was deemed appropriate for each trial, during examination, according to beetle activity.

For field trial 5.2, 35 logs were cut from a freshly harvested tree and established, using the same four replicate design as the first trial plus one extra replicate number five as shown in Figure 5.2. The first four replicates utilised the same compound sachets used in trial 5.1 (as they were meant to last six weeks), with new compound sachets used for replicate five. An additional replicate was included to ensure the effective duration of the sachets was six weeks. The billets were checked and the number of entry holes recorded every five days for a period of 25 days. At the completion of the trial, the bark was removed from each billet and adult beetles counted and sexed.

Field trial 5.3 was established using 44 billets 30 cm in length cut from a freshly harvested tree. The volatile compounds (A) *cis*-verbenol, (B) verbenone, (D) *trans*-verbenol, (E) α-pinene, (F) sulcatol, (G) MCH, and (H) MCOL were used. Five replicates were established in a circle configuration with a diameter of six metres. Each replicate contained three control billets. Each of the volatile compounds was replicated once in each of the five replicates. The exception was sulcatol, MCH, and MCOL which were only replicated once in three of the five replicates, due to the availability of less sachets of these compounds. So there were three replicates containing ten logs (seven treatments and three controls), and two replicates of seven logs (four treatments and three controls) as shown in

Figure 5.3. The billets were checked and the number of entry holes recorded every five days for a period of 42 days. At the completion of the trial, the bark was removed from each billet and adult beetles counted and sexed.

For field trial 5.4, one replicate was established involving both fresh and 'old' billets and fresh and 'old' MCOL sachets. The treatments were labelled A to F (A: Fresh billet plus new MCOL sachet; B: Fresh billet plus 'old' MCOL sachet; C: 'Old' billet plus new MCOL sachet; D: 'Old' billet plus old MCOL sachet; E: Fresh control billet; F: 'Old' control billet) and were established in the order shown in Figure 5.4.

The 'old' MCOL sachets were sachets which had been established in the field for a period of six weeks, which is their designed length of effectiveness. The 'old' billets plus MCOL were fresh billets 30 cm in length which had been established in the field for six weeks with MCOL sachets attached, so had *H. ater* occupation. The 'old' control billet was a fresh control billet which had been established in the field for six weeks. The fresh billets were cut from a mature *P. radiata* tree into billets of 30 cm length.

The billets were buried vertically in a circle approximately six metres in diameter with 1/3 of each billet above the soil surface.

The billets were examined every five days for a period of 21 days with the number of entry holes recorded. At the completion of the trial, the bark was removed from each billet and the adult beetles counted and sexed. The aim of this small trial was to determine how *H. ater* occupied billets in combination with MCOL which showed enhanced attraction by *H. ater* influenced attraction.

For field trial 5.5, 18 logs 30 cm in length were cut from a freshly felled *P. radiata* tree. Two replicates of nine billets were buried vertically with 1/3 above the soil surface in a circle approximately six metres in diameter. In each replicate of nine billets, three of the billets were controls and the remaining six billets had a compound sachet attached as previously described. The treatments were labelled A to G (A: *cis*-verbenol; B: verbenone; C: control; D: *trans*-verbenol; E: α-pinene; F: sulcatol; G: MCH). The treatments were in a random order within

each of the replicates while the controls maintained the same position as shown in Figure 5.5. The billets were checked and the number of entry holes recorded every five days for a period of 21 days. At the completion of the trial the bark was removed from each billet and numbers of *H. ater* or *H. ligniperda* beetles collected, counted and sexed.

The multiple funnel trap consists of a series of eight vertically aligned funnels with a collecting jar at the bottom (Lindgren 1983). Three funnel traps were established approximately one metre apart. The traps hung freely suspended on a large stake. The ipsenol and ipsdienol sachets respectively were attached to the inside of the collecting container at the bottom of two traps, with the third trap acting as a control. The traps were monitored at weekly intervals for a period of 12 weeks, with the sachets being replaced after the first six weeks. An additional funnel trap was also established for a period of six weeks with the compound frontalin being trialed.

Replicate 1							
Treatment	C	A	B	C	D	E	C
Replicate 2							
Treatment	C	A	D	C	E	B	C
Replicate 3							
Treatment	C	E	A	C	B	D	C
Replicate 4							
Treatment	C	B	D	C	E	A	C

Figure 5.1. Experimental lay out to assess potential activity of commercial compounds (Field trial 5.1)

Replicate 5							
Treatment	C	A	B	C	E	D	C

Figure 5.2. Experimental lay out to assess potential activity of commercial compounds (Field trial 5.2)

Replicate 1										
Treatment	C	A	B	F	G	C	D	E	H	C
Replicate 2										
Treatment	C	A	D	H	F	C	E	B	G	C
Replicate 3										
Treatment	C	E	A	G	H	C	B	D	F	C
Replicate 4										
Treatment	C	B	D	C	E	A	C			
Replicate 5										
Treatment	C	A	B	C	E	D	C			

Figure 5.3. Experimental lay out to assess potential activity of commercial compounds (Field trial 5.3)

Replicate 1						
Treatment	A	B	C	D	E	F

Figure 5.4. Experimental lay out to assess potential activity of commercial compounds (Field trial 5.4)

Replicate 1									
Treatment	C	A	B	C	F	G	C	D	E
Replicate 2									
Treatment	C	A	D	C	F	E	C	B	G

Figure 5.5. Experimental lay out to assess potential activity of commercial compounds (Field trial 5.5)

5.3.2.2. Laboratory bioassay of potential volatile compounds

The same commercial sachets of volatile compounds trialed in the field were tested for attractiveness to *H. ater* adult beetles in simple laboratory bioassays. The compounds *cis*-verbenol, *trans*-verbenol, verbenone, sulcatol, MCOL, MCH, frontalin, *exo*-brevicomin, verbenene, α-pinene, ethanol and *P.*

radiata bark were compared individually using a Y-tube olfactometer. The compounds MCOL and *cis*-verbenol were also tested in combination with α-pinene. The Y-tube olfactometer was connected to a small pump drawing air through the tubes at a flow rate of 100 ml/min as shown in Plate 5.1. Twenty adult beetles (ten male and ten female) were tested against each compound twice. First with the test compound placed on the right hand side of the Y-tube and then on the left hand side. The response of individual beetles was recorded as positive, negative, no response or backwards.

5.3.2.3. Role of *P. radiata* needles

A simple experiment was established to see if fresh pine needles played a role in the attraction of the black pine bark beetle. The experiment involved the use of pine needles wrapped in cardboard to resemble a *P. radiata* billet.

Pine needles were collected from a *P. radiata* tree which had just been harvested. The pine needles were removed from the stems and spread evenly over a piece of cardboard 40 x 60 cm in size. The cardboard was then rolled up tightly forming a cylinder with layers of pine needles and cardboard. The cylinders were then secured with heavy duty tape and the ends also sealed with the tape. The cylinders were then buried vertically with approximately 20 cm above the soil surface. Six cardboard cylinders were constructed, five with pine needles and one control. The cardboard cylinders were lifted on a weekly basis for a period of four weeks and examined for *H. ater* invasion.

5.3.2.4. Role of *P. radiata* bark

Two trials were established to determine if there were any factors in the pine bark which could result in the attraction of *H. ater* to other tree species wrapped in a layer of *P. radiata* bark (including cambium). Billets of *Acacia* and *Eucalyptus* spp. were offered to *H. ater* for invasion.

Trial 5.6 involved bark being removed from freshly felled *P. radiata* billets and wrapped around *Acacia* and *Eucalyptus* spp., of the same 40 cm length

and fastened with heavy duty tape. The billets were then buried vertically with approximately 10 cm of the billet above the soil surface. Three replicates of each treatment were buried along with three controls of each tree species. The billets were checked for invasion at weekly intervals over a period of eight weeks.

Trial 5.7 was established using the same method as described for trial 5.6, except that only *Acacia* spp. billets were used. Four pine billets and four wattle billets were used as controls, while four wattle billets were wrapped in pine bark, and another four wattle billets were wrapped in pine bark with a layer of paper towel soaked in sugar solution in between the bark. The sugar solution treatment was included to assist maintenance of bark moisture content. All billets were examined at weekly intervals for *H. ater* invasion over a period of eight weeks.

5.3.2.5. Role of *P. radiata* chemicals

Various sources of *P. radiata* volatiles were trialed using simple bottle and bark resemblance traps as shown in Plates 5.2 and 5.3 respectively to establish if they were attractive to *H. ater*. The solutions used included the *P. radiata* host volatile α-pinene, and blackcurrant solution as a source of sabinene; the by products of a ferment process including a sugar ferment solution and a pine needles sugar ferment solution as a source of ethanol, vinegar as a source of acetic acid, and paraldehyde as a condensed acetaldehyde; phenyl; and distilled water. These solutions were chosen as they either resemble or are naturally occurring compounds in *P. radiata* or result from biological breakdown processes.

Trial 5.8 of the bottle attraction traps was established at three sites comprising three replicates of the four treatments: sugar-ferment solution; distilled water; sugar-ferment solution plus 5% α-pinene; and 5% α-pinene.

The sugar-ferment solution consisted of 666.6 g sucrose, 6.6 g KNO_3, 2.0 g $MgSO_4$, 2.0 g KH_2PO_4, 2.0 g yeast extract, and 1000 ml water. The dry ingredients were dissolved in water one day prior to use in the field. The α-pinene solution was made up in hexane, and 10 ml applied to a cotton pad, and attached to the outside of the bottles.

At each of the sites the following method was employed. Once the treatments were placed in the bottles, a piece of gauze was attached to the top of the bottle to prevent anything from getting in, and a piece of sisalation paper placed over the top like an umbrella to prevent rain from entering. Wooden stakes were inserted into the ground and the 375 ml bottles containing the respective treatments were placed at the base of the stakes and cylinders of 'fly-wire' mesh coated in 'Stick-Um' placed over the stake and bottle to keep the bottle in place.

The mesh had previously been prepared in the laboratory where 'Stick-Um' was softened in the microwave and then painted onto the mesh sections (30 x 25 cm) and a piece of plastic sheet placed between each layer so that in the field each piece of mesh could be peeled off the plastic and the mesh then stapled to form a cylinder of precoated mesh.

The traps were checked four and ten days after establishment and then at weekly intervals for a period of eight weeks.

In trial 5.9 six solutions were used including: 10% α-pinene in hexane; sugar-ferment solution; vinegar; 10% paraldehyde; phenyl; and distilled water. The same three sites as trial 5.8 were selected, with each treatment replicated three times (once at each site), except for the α-pinene solution and phenyl which were only replicated twice (once each at only two of the sites). The experiment was established just the same as trial 5.8, except that wicks were placed in each bottle instead of the gauze covering being used. The wicks were approximately 30 cm long made from cotton wool and each wrapped in cotton cloth material and stapled closed along the edge.

Trial 5.10 was established using the same method as trial 5.9, with the same six solutions being replicated at each of the three sites.

Four bark resemblance attraction traps were established at different sites. A tin (20 cm high, 15 cm diameter) was buried in the soil flush with the soil surface and a 26 cm high bottle covered in towelling material was inverted in the tin, and a solution added to the tin to soak upwards in the towelling. Approximately 500 ml of a 10% paraldehyde solution was added to the tins and

replaced at monthly intervals. The paraldehyde solution was trialed as previous work had resulted in the attraction of large numbers of *H. ater* to one of two paraldehyde bark resemblance attraction traps. The traps were checked on a weekly basis over the four months that they were established. Two water control bark resemblance attraction traps were also established.

In addition to the paraldehyde traps, two other solutions, a blackcurrant and a pine needle sugar solution were also trialed over a four week period.

The blackcurrant solution was trialed as blackcurrants contain high levels of sabinene. During volatile extraction work sabinene was found to be a compound present within *H. ater* following a period of feeding.

The pine needle sugar solution was made by dissolving one kg of sugar in water to which pine needles were added and allowing the pine needles to steep for a period of two weeks in a 25°C incubator.

5.3.2.6. Role of α-pinene

The primary monoterpene, α-pinene the major monoterpene present in *P. radiata*, was applied to non host material to determine its possible involvement in the attraction process.

Twelve freshly harvested *Acacia* spp. billets 60 cm in length were buried vertically with 20 cm above the soil surface. Four of the billets acted as control billets. Four billets were coated in α-pinene and four billets were coated in 'Stick-Um' and then α-pinene. The α-pinene was applied at 100% concentration. The billets were examined each month for a three month period, being recoated after each inspection.

5.3.3. Analysis of Feeding Beetles and Frass

A 4.0 x 2.0 x 0.2 cm piece of *P. radiata* bark and phloem (approximately 2.0 g) was placed in each of six glass vials (vial dimensions 5.0 x 2.5 cm) with 'push on' plastic lids which sealed, with an equal number of small holes made in

the top of each lid to enable ventilation. The bark and phloem samples were taken from a freshly harvested *P. radiata* billet.

All six vials were analysed using GC-MS on day 0 when the bark and phloem samples were placed in the vials, after which 12 *H. ater* beetles (six female and six male) were placed in three of the vials labelled treatments 1, 2, and 3, with the remaining three vials acting as control 1, 2, and 3. The vials were then analysed again on day 2, 4, 6, 10, 15, 21 and 24.

A Supelco solid phase microextraction (SPME) syringe with a 100 μm polymethylsiloxane fibre, was inserted through each vial lid and the fibre exposed for a collection period of 10 min. Analysis was by combined GC-MS of HP 5890 Gas Chromatograph coupled to a HP 5970B mass selective detector. Desorption of the syringe was for a period of five min onto a HP-1 column (25 m x 0.32 mm i. d., 0.52 μm film thickness). The injector temperature was 250°C, the GC-MS interface temperature 290°C and the GC oven was programmed to operate from 30 to 250°C, increasing at 10 °C/min after an initial hold at 30°C for five min. Electron impact mass spectra were acquired at 70 eV, scanning from *m/z* 40 to 330 at a rate of 1.25 scans per sec.

On day 24 following the final GC-MS analysis, all beetles were removed from the treatment vials and the resultant frass pooled together and analysed. The 0.99 g of frass was then used for a solvent extraction with chloroform, involving addition of solvent, 10 minutes sonication, separation of solvent, repeated three times. The solvent was then evaporated leaving approximately 1.5 ml of extract. The resulting extract was only just detectable when run through the GC-MS, so 40 vials of bark and phloem and beetles were established in the same manner as described previously. The 4.24 g of accumulated frass during more than four weeks of feeding was then used in a soxhlet extraction with hexane and subsequently analysed.

5.3.4. Analysis of *P. radiata* Material

Old and fresh *P. radiata* materials were extracted via a cold pentane extraction at 10°C for various lengths of time from 16 hours to three days, and then analysed by GC-MS as described for the insect volatile extraction samples.

Three duplicate pairs of *P. radiata* billets were cut from a freshly harvested tree, with one set of each pair placed at -20°C and the other set placed at 4°C. On day 0 the billets were removed from the freezer and fridge and left at room temperature. A cork borer of diameter 2.0 cm was used to remove a section of bark and phloem from each billet on day 0, 3, 7, 18, and 22. The samples were analysed using GC-MS as described for the analysis of feeding beetles.

Three *P. radiata* billets were cut from a freshly harvested tree, as well as three billets from a dry *P. radiata* billet which had been felled for more than twelve months. Bark and phloem discs 2.0 cm in diameter were removed from the billets and analysed using GC-MS as described for the analysis of feeding beetles. The discs were placed in glass vials and covered in water and allowed to soak for a period of six weeks and then analysed again.

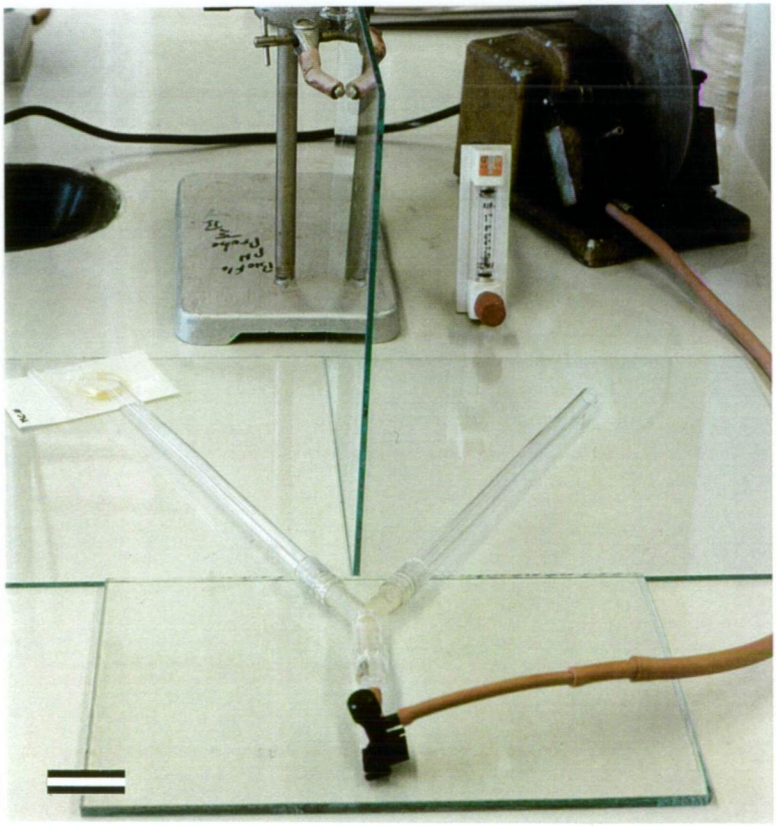

Plate 5.1. Y-tube olfactometer apparatus (Bar = 3 cm)

Plate 5.2. Bottle attraction trap (Bar = 3 cm)

Plate 5.3. Bark resemblance attraction trap (Bar = 4 cm)

5.4. RESULTS

5.4.1. Insect Volatile Extraction

The initial GC-MS analysis of the whole beetle extracts detected the presence of low levels of volatiles with only two *P. radiata* host derived volatiles being present. The two standard markers 2- and 3-octanol were evident and only trace amounts of α- and β-pinene were detected.

The GC-MS analysis using SIM for monoterpene based compounds enabled the detection of volatiles from the whole beetle extracts, although the detected monoterpenes were still present in low levels. An example of a gas chromatogram achieved from the analysis of whole beetle extracts from a sample of four pooled fed female beetles is shown in Figure 5.6.

The compounds isolated from emergent non-feeding adult *H. ater* beetles and beetles which had fed for various lengths of time are shown in Table 5.1, along with the compounds which were recorded in the host substrate, *P. radiata*.

An example gas chromatogram of the headspace analysis is shown in Figure 5.7. A large number of compounds were detected which were different from the volatiles detected from the beetle extractions, including n-pentanal, heptane, n-pentanol, n-hexanal, n-octane, n-hexanol, n-heptanal, n-heptanol, n-nonanal, dec-2-enal, deca-2, 4-dienal, 2-undecenal, unsaturated aliphatic, and C9 and C10 unsaturated aldehydes. Many of these compounds are associated with alarm pheromones, being released by the beetles under the stress conditions of being placed in a glass vial.

	H. ater emergent	*H. ater* fed	*P. radiata*
α-pinene	+	+	+
β-pinene	+	+	+
β-phellandrene	+	+	+
δ-3-carene	+	+	+
Limonene	+	+	+
Myrcene	+	+	+
Sabinene	-	+	+
Terpinolene	+	+	+

Table 5.1. Compounds isolated from emergent and fed adult *H. ater* beetles and *P. radiata* host material

5.4.2. Assessment of Potential Volatile Compounds

5.4.2.1. Field trials

As shown in Figure 5.8 for trial 5.2 and Figure 5.9 for trial 5.3, *H. ater* were attracted to the *P. radiata* billets and associated volatile compound treatments within the first seven days of establishment with a peak being reached which then declined. This general trend was evident in all field trials.

None of the volatile compound treatments were shown to be significantly different statistically for any of the volatile compound field trials when compared on the basis of number of entry holes, number of beetles and number of male and female beetles per cm^2 and cm^3 of *P. radiata* material except for trial 5.3, where MCOL was significantly different (Appendices 9, 10, 11 and 14). The strong attraction to MCOL was evident from establishment as shown in Figure 5.9. For trial 5.3 the MCOL treatment was shown to be just significantly different with a p-value of 0.0479 when compared on the basis of *H. ater* entry holes per cm^3 of *P. radiata* material and marginally significantly different with a p-value of 0.0884 when compared on the basis of *H. ater* entry holes per cm^2 of *P. radiata* material (Appendix 11).

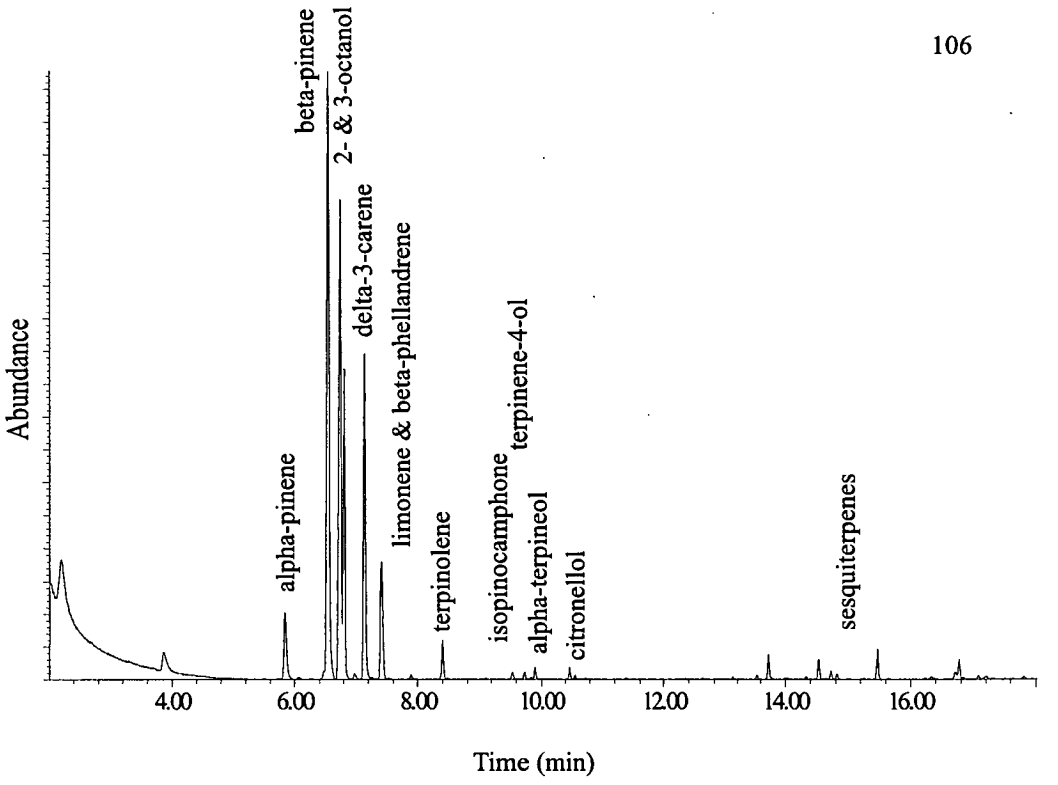

Figure 5.6. Gas chromatogram of adult H. ater whole beetle extraction of four pooled female beetles which had undergone five weeks of feeding

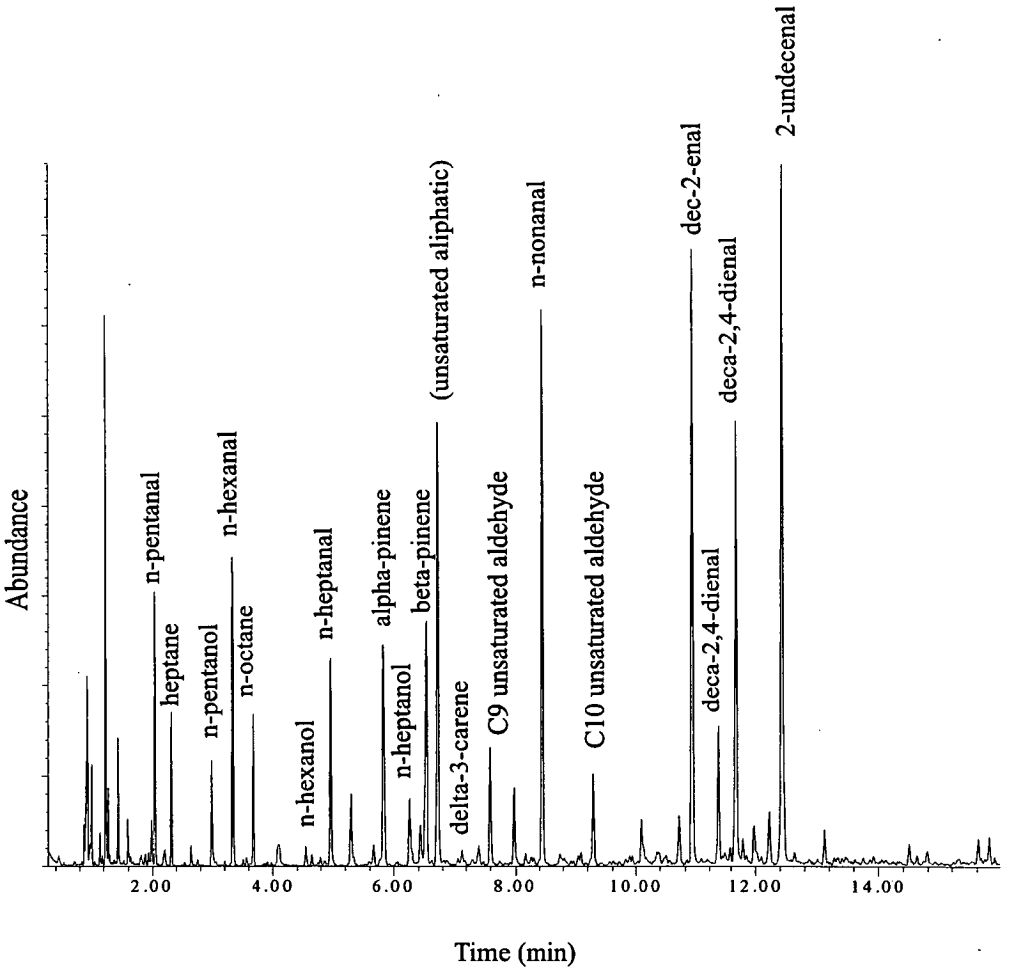

Figure 5.7. Gas chromatogram of H. ater headspace analysis

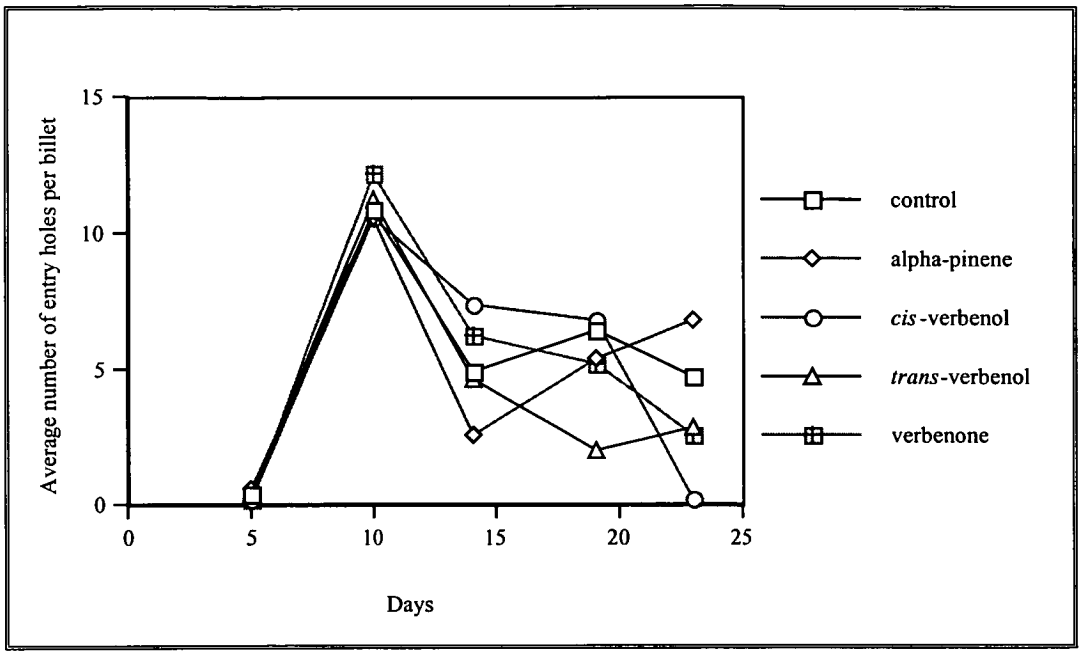

Figure 5.8. Average number of *H. ater* entry holes per *P. radiata* billet over time for assessment of potential activity of volatile compounds (Field trial 5.2) (Appendix 12)

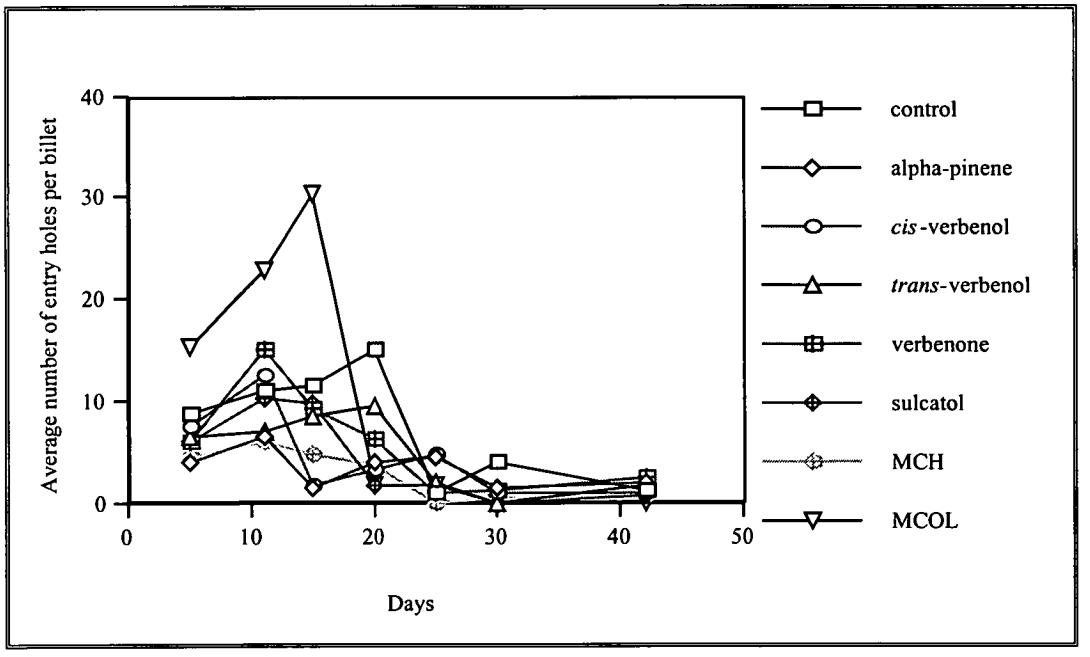

Figure 5.9. Average number of *H. ater* entry holes per *P. radiata* billet over time for assessment of potential activity of volatile compounds (Field trial 5.3) (Appendix 13)

The number of *H. ater* and *H. ligniperda* beetles and other insects caught over the twelve weeks that the funnel traps were monitored are shown in Table 5.2. The funnel traps were ineffective in capturing *H. ater* beetles. This is presumably due to the compounds trialed being unattractive, and also the flying characteristics of the beetle, which would be expected to be low to the ground in search of their host material. The other insects caught in the funnel traps included the beetle families Scarabaeidae, Cantharidae, Elateridae, and Carabidae, as well as members of the families Blattidae and Kalotermitidae.

Trap	Total *H. ater*	Male	Female	Total *H. ligniperda*	Male	Female	Other Insects
Ipsenol Funnel trap	2	2	0	14	7	7	27
Ipsdienol Funnel trap	2	1	1	11	3	8	30
Frontalin Funnel trap	0	0	0	0	0	0	15
Control Funnel trap	1	1	0	0	0	0	10

Table 5.2. Total number of insects caught with the funnel traps

5.4.2.2. Laboratory bioassay of potential volatile compounds

The responses of adult *H. ater* beetles to all volatile compounds trialed in laboratory bioassays were shown to be statistically non-significant when compared on the basis of compound and beetle sex (Appendix 15). The only significant response was that to *P. radiata* bark where 100% positive response was recorded by both *H. ater* male and female beetles.

5.4.2.3. Role of *P. radiata* needles

No *H. ater* invasion (or any other insect attack) was noted throughout the four week period that the pine needles cardboard cylinders were checked on a weekly basis.

5.4.2.4. Role of *P. radiata* bark

Trial 5.6 resulted in only a total of eight entry holes into the pine bark wrapped around all six wattle and eucalypt billets. There was no entry into any of the wattle or eucalypt control billets.

Trial 5.7 resulted in a much higher invasion of *H. ater* as shown in Table 5.3. No beetles entered the wattle control billets, although all of the wattle billets wrapped in pine bark showed entry holes.

Treatment	Total Number Entry Holes	Number Entry Holes/Billet ± SE
Wattle/Pine/Sugar Solution Billet	30	7.5 ± 1.3
Wattle/Pine Billet	9	2.3 ± 0.5
Pine Control Billet	58	14.5 ± 4.7
Wattle Control Billet	0	0

Table 5.3. Number of *H. ater* entry holes recorded on wattle billets wrapped in *P. radiata* bark (Trial 5.7)

5.4.2.5. Role of *P. radiata* chemicals

No *H. ater* were present on any of the traps in the first and second trial of the bottle attraction traps (Trials 5.8 and 5.9). Strong winds following establishment of the third trial 5.10 had blown nearly all the bottles, wicks and mesh away at all of the sites, so the third trial was destroyed.

No *H. ater* beetles were attracted to either the blackcurrant solution or the pine needles sugar solution in the bark resemblance attraction traps. Only a total of 12 *H. ater* beetles were found in all of the paraldehyde traps over the entire four months that the solutions were maintained, with no beetles present in the water control traps.

5.4.2.6. Role of α-pinene

No evidence of *H. ater* invasion by the presence of entry holes were found on the untreated wattle control billets or the wattle billets coated in only α-pinene. Adult *H. ater* beetles were only recorded on the wattle billets coated in α-pinene

and 'Stick-Um' as shown in Table 5.4, with attracted beetles being trapped in the 'Stick-Um'.

Treatment	Total Number *H. ater*
Wattle Control	0
Wattle billet & α-pinene	0
Wattle billet & α-pinene & 'Stick-Um'	20

Table 5.4. Total number of *H. ater* beetles attracted to wattle billets coated in α-pinene

5.4.3. Analysis of Feeding Beetles and Frass

The Supelco SPME syringe was successful in collecting the volatiles present within the sealed glass vials containing *P. radiata* material and feeding beetles. The SPME syringe enabled the collection of volatiles from attacking beetles under near-natural conditions. During initial introductory experiments exposing the syringe to *P. radiata* billets containing feeding beetles in an open laboratory was unsuccessful due to the collection of numerous external contaminant materials. The adult beetles appeared content in the sealed vials with the production of large quantities of frass over time. Gas chromatograms of one of the treatment and control vials at day 0 and day 24 are shown in Figures 5.10 and 5.11 respectively.

Almost all of the compounds isolated from both the control and treatment vials were monoterpenes or monoterpene based. The analysis of the bark and phloem on day 0 showed the presence of the main host monoterpenes α-pinene, β-pinene, myrcene, δ-3-carene, limonene, β-phellandrene and terpinolene. Over the duration of the analysis these main monoterpenes in the treatment and control vials showed a general decline as shown in Figures 5.12a and 5.13a. The use of the SPME syringe was not intended to be a quantitative technique although the general trends detected in both treatment and control vials were replicated in all vials. The total ion current depicted in the graphs does not represent absolute amounts so the technique is not strictly quantitative.

The feeding beetle samples contained the same compounds as the control samples as well as numerous oxidised monoterpenes such as camphor, fenchone, myrtenal, terpinene-4-ol, isopinocamphone, and verbenone. Fenchone showed a general increase detectable four to six days after feeding commenced, being only present in trace amounts in the control vials. Isopinocamphone also increased over time in both the control and treatment vials. Camphor increased over time although showing some fluctuation being present in trace amounts at day 0 in both control and treatment vials. Figures 5.12b and 5.13b show these general declines for the feeding beetle and the control samples respectively. Analysis of the frass showed that these compounds were coming from the frass as shown in the gas chromatogram of *H. ater* frass shown in Figure 5.14.

An unknown compound of molecular weight 140 was detected in the treatment vials four days after feeding commenced and continued to increase over time. This compound was not present in the control vials. The unknown was further analysed by GC combined with high resolution MS on a Kratos Concept ISQ instrument, using perfluorokerosene as an internal mass calibrant. The mass spectrum was determined as M^+ 140 (17%), 125 (51), 71 (100), 70 (17), 69 (31), 68 (31), 67 (22), 55 (49), 53 (21), 43 (43), 42 (36), 41 (51). The masses were measured as 140.1194 ($C_9H_{16}0$ requires 140.12012), 125.0977 ($C_8H_{13}0$ requires 125.9664) and 71.054 (C_4H_70 requires 71.04969).

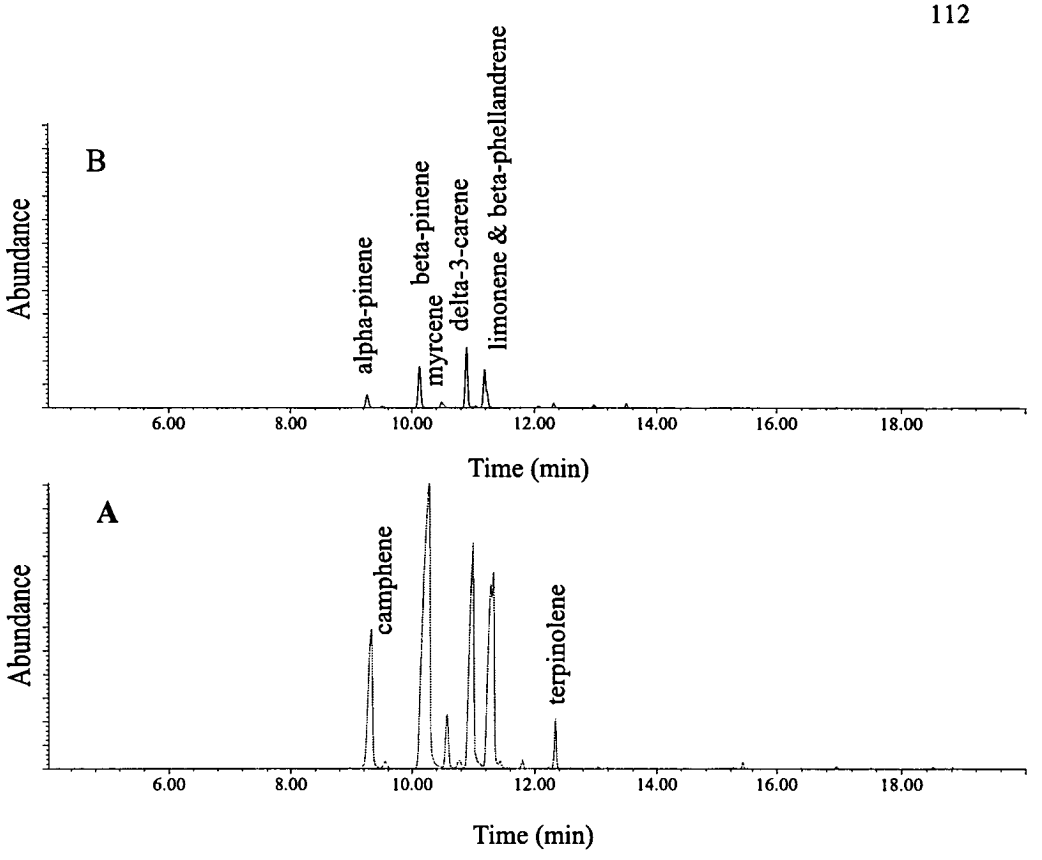

Figure 5.10. Gas chromatogram of H. ater adult beetles feeding on P. radiata material at A) Day 0 and B) Day 24

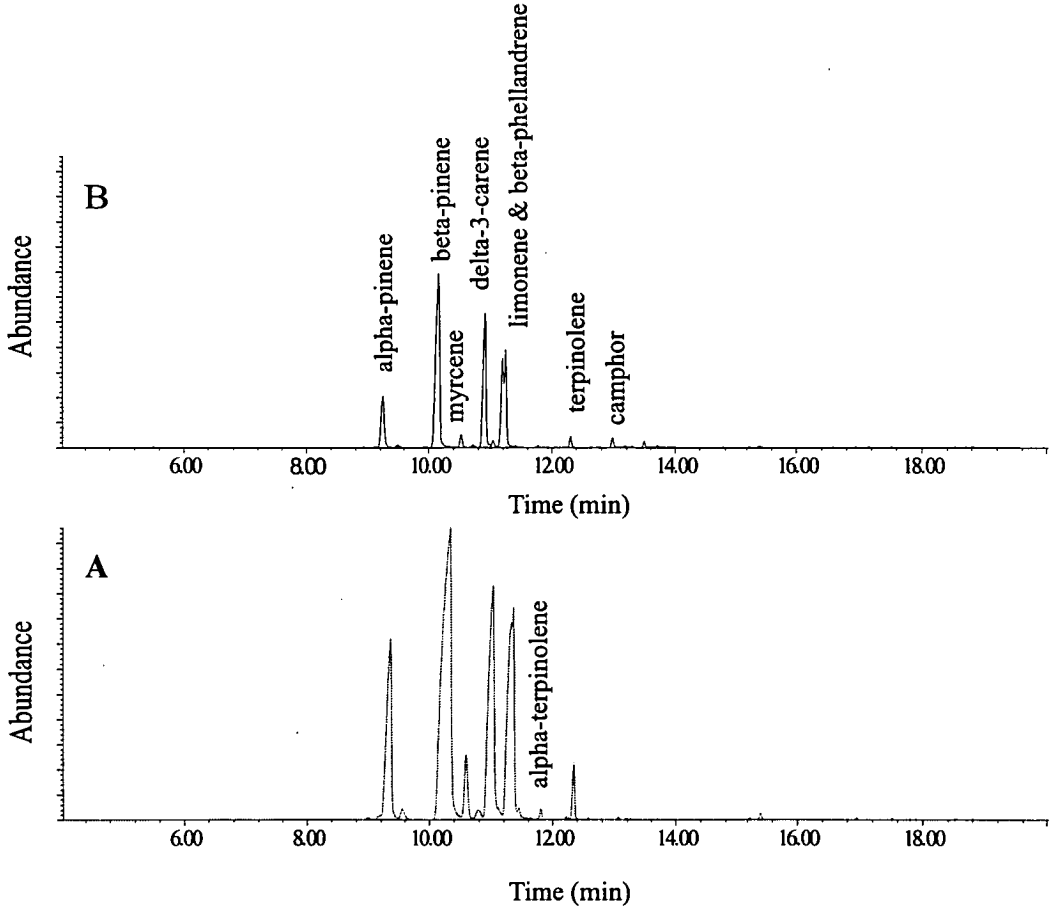

Figure 5.11. Gas chromatogram of P. radiata material at A) Day 0 and B) Day 24

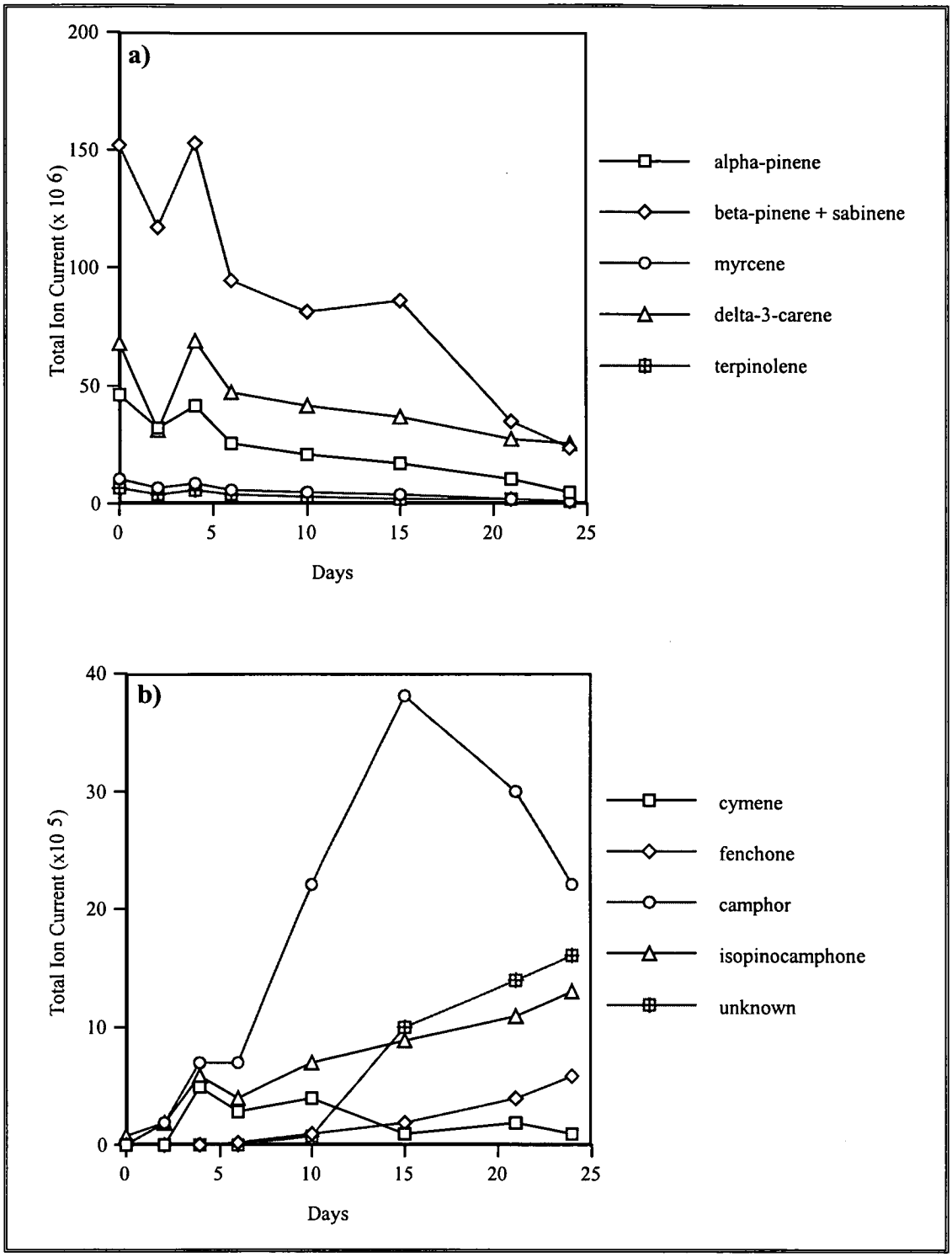

Figure 5.12. Volatile compounds detected from feeding *H. ater* beetles on *P. radiata* over time (Appendix 16)

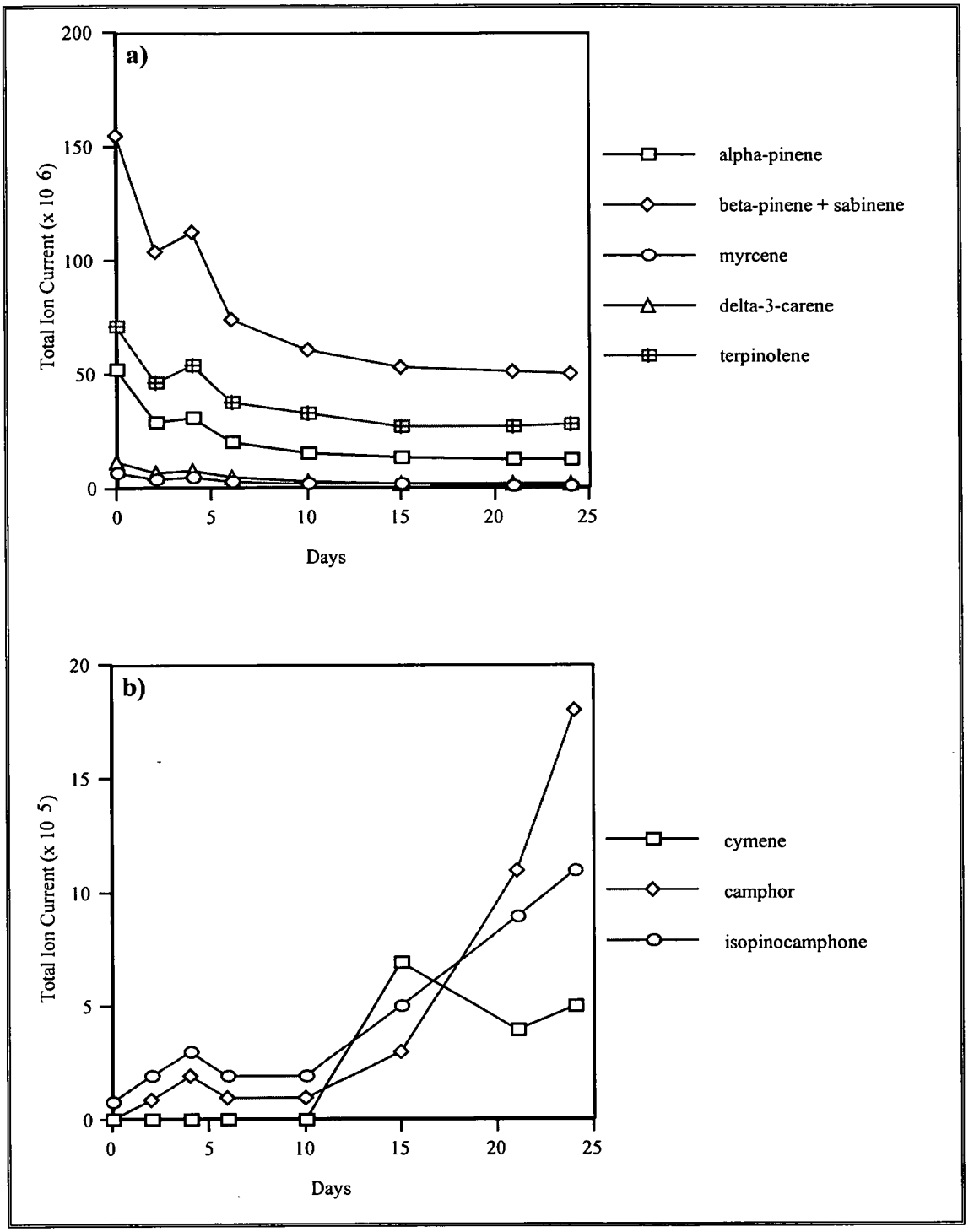

Figure 5.13. Volatile compounds detected from *P. radiata* over time (Appendix 16)

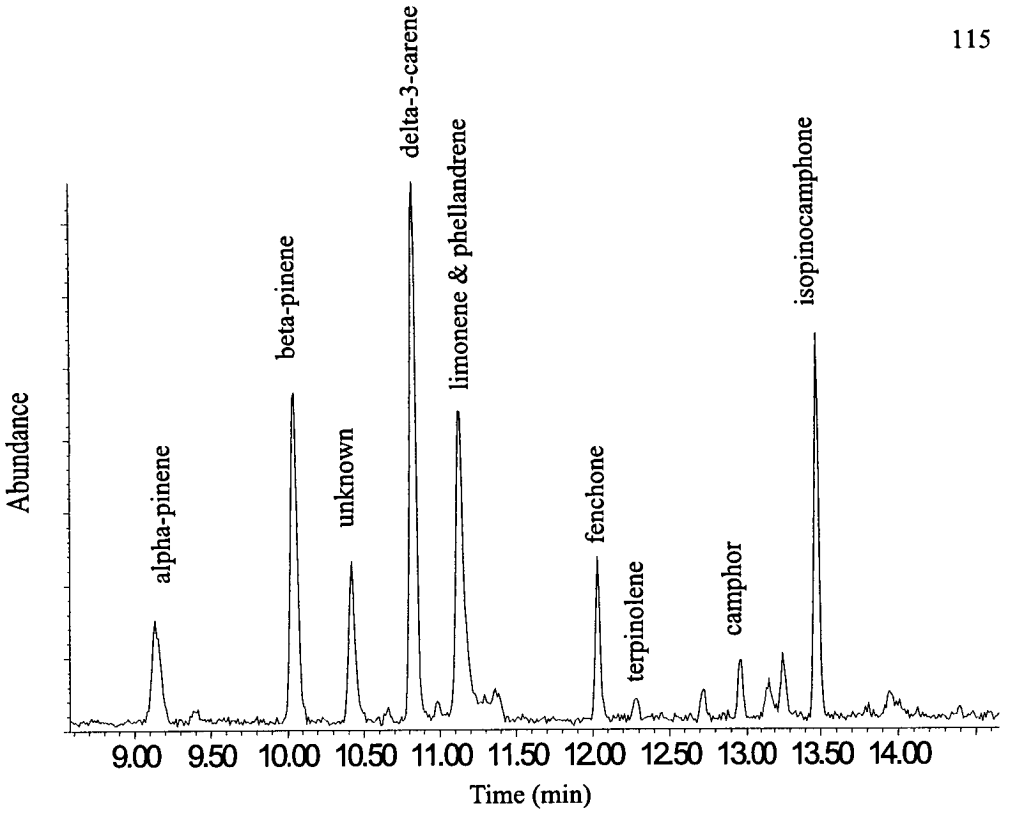

Figure 5.14. Gas chromatogram of H. ater frass collected from beetles feeding on P. radiata material for 24 days

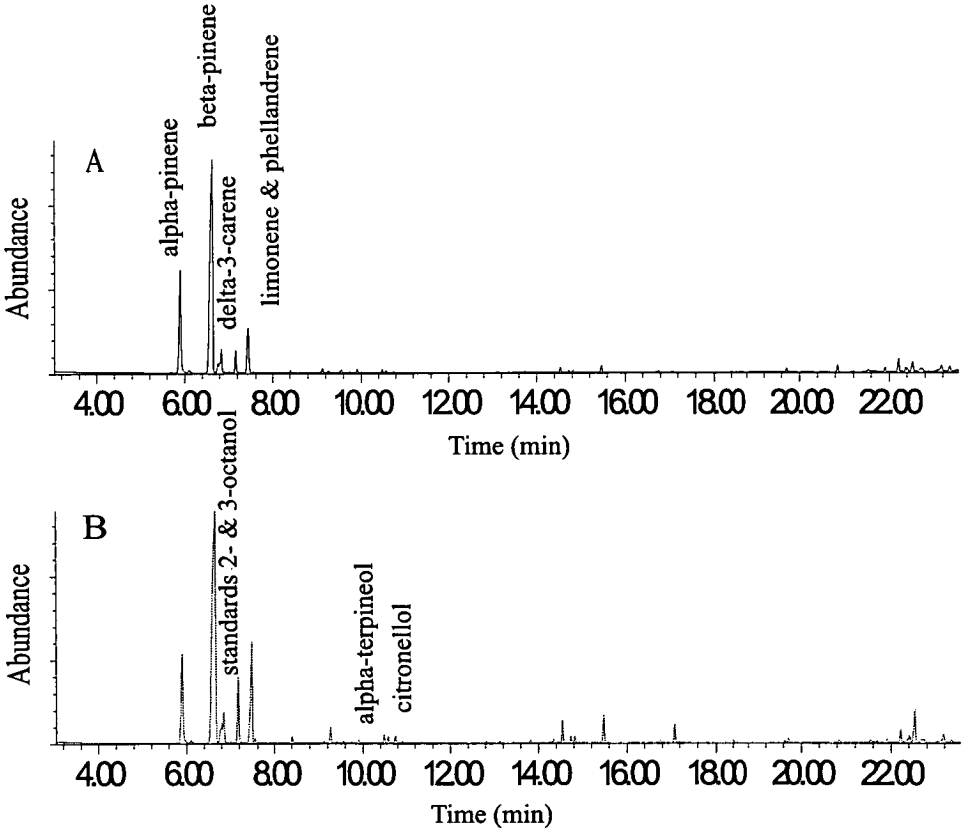

Figure 5.15. Gas chromatogram of A) dry and B) fresh P. radiata bark and phloem

5.4.4. Analysis of *P. radiata* Material

It is important to re-emphasis that this technique is not quantitative, but general trends were detected. The GC-MS analysis of the pentane bark/phloem extracts detected the same main monoterpenes in dry ('old') *P. radiata* bark as detailed for fresh *P. radiata* bark in Table 5.1. The main difference being a general reduction in level of compounds in dry *P. radiata* material. Example gas chromatograms of the pentane bark extracts for dry and fresh *P. radiata* bark and phloem are shown in Figure 5.15.

The compounds detected in fresh and dry *P. radiata* bark and phloem which had been subjected to a water soaking treatment and damaged by freezing are summarised in Table 5.5.

Compound	Fresh *P. radiata* billet	Fresh Soaked *P. radiata* billet	Dry *P. radiata* billet	Dry Soaked *P. radiata* billet	Frozen Fresh *P. radiata* billet
α-pinene	++	+	++	+	+++
camphene	+	+	+	+	+
β-pinene	++++	++	++	+	+++
myrcene	+	+	+	+	+
δ-3-carene	+	+	+	+	+
cymene	-	-	+	+	+
β-phellandrene + limonene	++	+	+	+	++
fenchone	-	-	+	+	+
terpinolene	+	+	+	+	+
camphor	-	+	+	+	+
pinocarveol	-	-	+	-	+
pinocamphone	-	-	+	+	+
isopinocamphone	-	-	+	+	+
terpinene-4-ol	-	-	+	-	+
α-terpineol	-	+	+	+	+

Table 5.5. Compounds from *P. radiata* bark/phloem extracts that were detected with GC-MS analysis

The same compounds were detected in both dry and fresh *P. radiata* material before and after water soaking. The main difference being a general decline in the level of compounds after soaking and the detection of oxidised monoterpenes in dry *P. radiata* material. Similar compounds detected in both fresh and dry *P. radiata* material was expected. The detection of oxidised

monoterpenes in dry material is characteristic of the conversion of the main monoterpenes and in turn their decline. Example gas chromatograms of fresh and dry *P. radiata* material before and after water soaking are shown in Figures 5.16 and 5.17 respectively.

GC-MS analysis of damaged *P. radiata* material by freezing detected the presence of the main monoterpenes as well as the emergence over time of the oxidised monoterpenes including camphor, terpinolene, fenchone, pinocamphone, isopinocamphone, terpinene-4-ol, verbenone, isopinocamphone, and fenchone. A gas chromatogram of *P. radiata* material which had been subjected to freezing and then thawing for 18 days is shown in Figure 5.18.

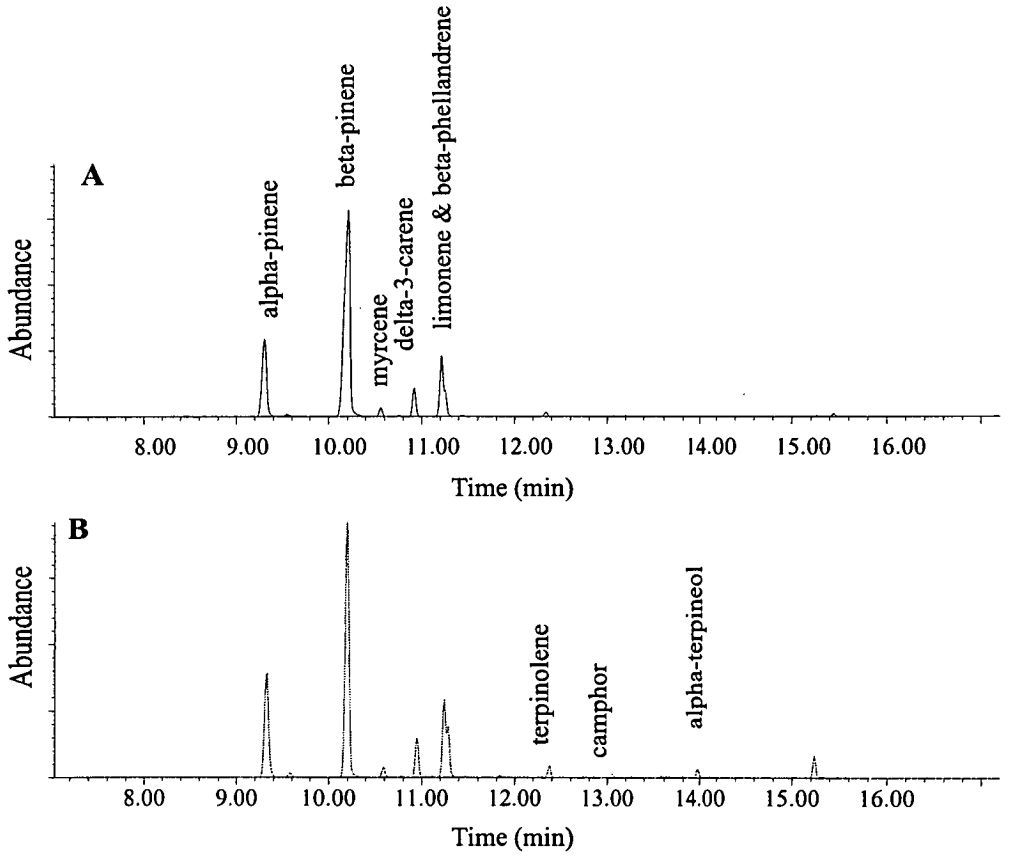

Figure 5.16. Gas chromatogram of A) fresh P. radiata bark and B) soaked fresh P. radiata bark

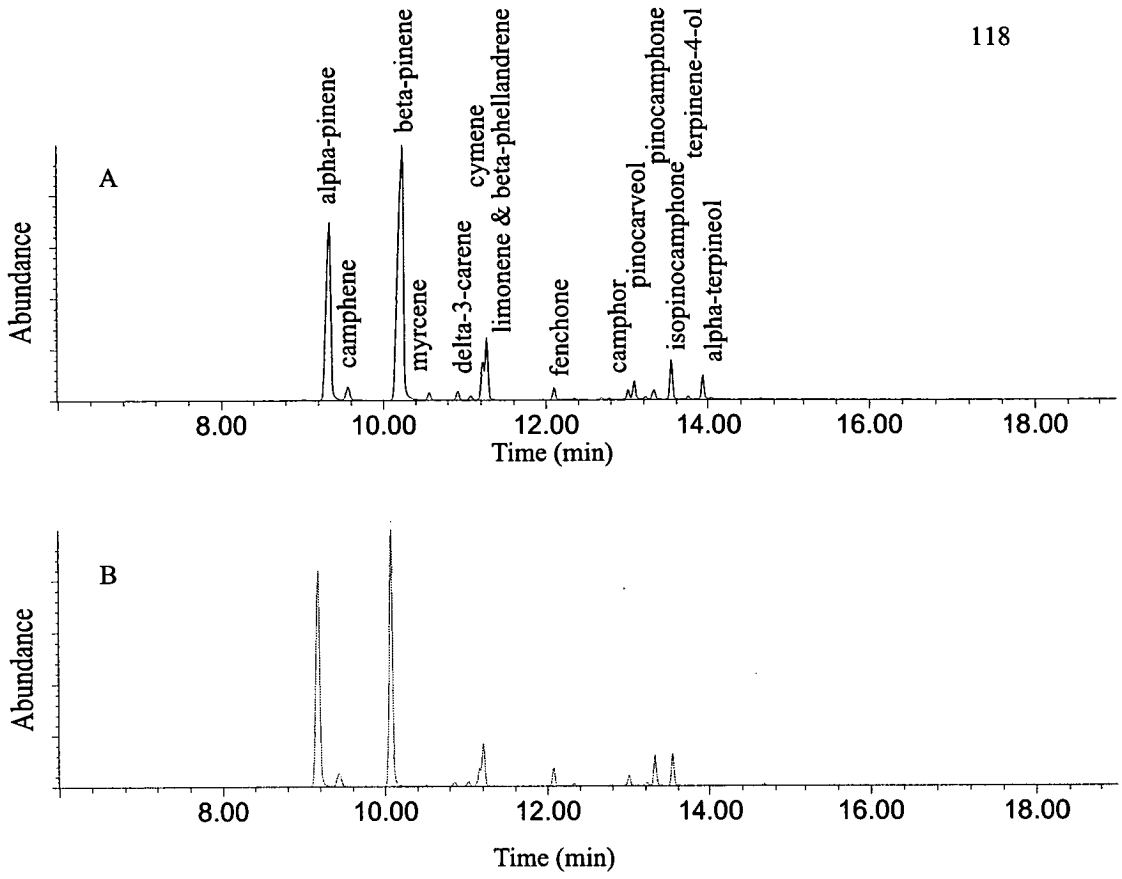

Figure 5.17. Gas chromatogram of A) dry P. radiata bark and B) soaked dry P. radiata bark

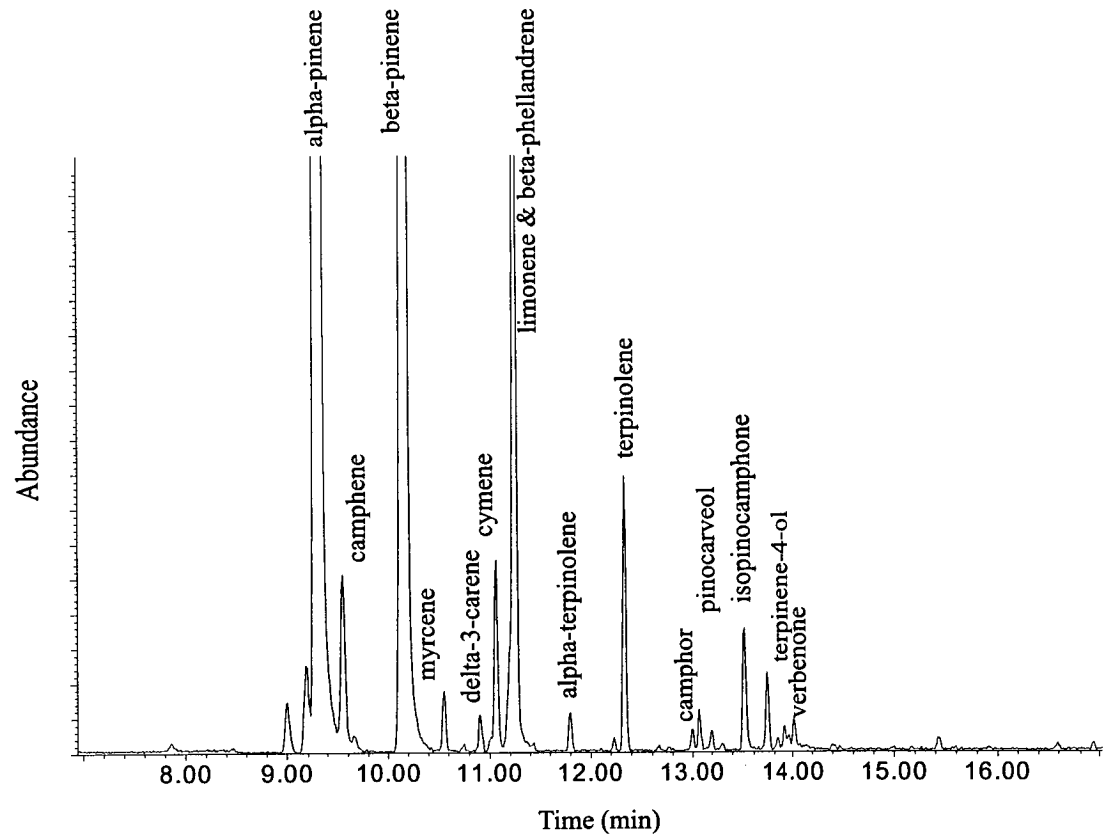

Figure 5.18. Gas chromatogram of thawed frozen P. radiata bark after 18 days

5.5. DISCUSSION

5.5.1. Insect Volatile Extraction

The quantities and number of compounds detected within *H. ater* were considerably lower than found in similar work conducted with other bark beetle species (Renwick 1967, Pitman et al 1968, Kinzer et al 1969, Pitman and Vite' 1970, Vite' et al 1972, Rudinsky et al 1974, Madden et al 1988). However, most volatile extraction literature involves primary bark beetles.

Monoterpene compounds were present in detectable levels but not enough to enable quantity determinations. The general quantity of monoterpene compounds isolated from *H. ater* varied between emergent non-feeding adults and beetles which had fed for various lengths of time with the general level of detected monoterpenes being higher in fed beetles.

All of the compounds isolated from adult beetles which had fed were present within the *P. radiata* host. This study showed that large genetic differences exist within *P. radiata* with the detected monoterpenes varying between sources. Not all of the monoterpenes listed in Table 5.1 were detected in all *P. radiata* material.

The main monoterpene hydrocarbons detected in *P. radiata* were the same as those identified by Simpson and McQuilkin (1976). The GC-MS analysis of *P. radiata* extracts detected the main monoterpenes α-pinene, β-pinene, β-phellandrene, δ-3-carene, limonene, myrcene, sabinene and terpinolene.

An interesting finding was that of sabinene when present in *P. radiata* material was only detected as a trace amount not fully resolved, being incorporated into the β-pinene peak, but when detected in the beetle extracts sabinene was fully resolved as a distinct peak with the other monoterpenes. Sabinene was detected in both male and female adult beetles following a period of feeding. Sabinene is the main constituent (~30%) of the oil of savin from the Savin Juniper, *Juniperus sabina* L. (Cupressaceae). The oil of savin is poisonous and has been used as an insect repellent (Everett 1981).

The pentane extraction method employed was effective but the analysis showed that fats and residues were also being extracted as they contaminated the GC column.

Even though specifically looked for, none of the 113 beetles processed and analysed showed any major compound separate to the detected host monoterpenes which may act as an attractant or repellent in *H. ater* host selection and colonisation stages. The habitat and host material utilised by the secondary bark beetle, *H. ater* differs from most primary bark beetles for which considerable semiochemical research has been conducted, for example members of the family *Dendroctonus*. *H. ater* is a soil dwelling species primarily attacking logging residues while *Dendroctonus* spp. attack living trees above the ground. To enable successful colonisation of living trees such primary bark beetles need to aggregate in sufficient numbers to overcome host resistance. Following initial discovery of the host by a few pioneer beetles insect produced compounds combine with host produced compounds to induce and regulate the mass aggregation and attack (Borden 1982). As *H. ater* is a soil dwelling species which does not attack mature living trees they have no need for insect produced compounds to assist the aggregation and attack stages of host selection. *P. radiata* host monoterpenes act as the primary attractant in *H. ater* host selection with beetles being able to discriminate between suitable host material and that which is unsuitable. Primary attraction has been exhibited by two *Hylastes* spp., *H. gracilis* (Miller et al 1986) and *H. nigrinus* (Borden 1977). *Hylastes* spp. have been known to respond to plant produced attractants in the form of volatile materials (Vite' & Pitman 1967).

The headspace trials collected different volatiles than were detected from the beetle extractions, presumably due to the limited space in the 2 ml vials which would have induced stress conditions for the beetles. This was assumed to be different to what happens in nature being in a 2 ml vial resulting in the collection of a number of compounds which were not detected in later analyses of feeding beetles not exposed to stress conditions.

5.5.2. Assessment of Potential Volatile Compounds

5.5.2.1. Field trials

None of the compounds tested in the field trials, induced any more beetle invasion by entry holes or actual beetles, than the control logs and all treatments were shown to be statistically non significant. The average number of *H. ater* which entered the billets was fairly constant for all treatments in trials 5.1 and 5.2. The mean number of entry holes per cm^2 for each treatment only varied between 0.009 and 0.007 for trial 5.1 and 0.025 and 0.018 for trial 5.2.

From the first trial (5.1), it was thought that the billets needed to be monitored more closely than on a weekly basis. The second trial (5.2), gave a better representation over time of the invasion pattern which was replicated in subsequent trials. The most activity occurs between five to ten days after establishment, with the maximum number of beetles invading during this time. This was followed by a decline with little activity after 25 days as shown in Figures 5.8 and 5.9.

In trial 5.3, billets associated with MCOL were invaded by more *H. ater* than any of the other volatile treatments from the first recording, with a peak response reached after 15 days which then declined. These changes are depicted in Figure 5.9. On the basis of *H. ater* entry holes per cm^3, there was a marginal significant treatment difference of the MCOL compound compared to the other treatments with a p-value of 0.0479 (Appendix 11).

Trial 5.3 showed the same pattern as the other trials, with none of the compounds gaining a response greater than the control billets except for MCOL. These results suggest that MCOL may act as an attractant as part of the host selection and colonisation stages of *H. ater*. MCOL acts as an aggregation pheromone for the Douglas-fir beetle, *D. pseudotsugae* (Rudinsky et al 1972, Lindgren et al 1992).

The number of *H. ater* found in the funnel traps were low, with only five *H. ater* adult beetles caught on the traps throughout their establishment. A total of 25 *H. ligniperda* were captured by the ipsenol and ipsdienol treatments. The

funnel traps did not appear to be a successful method of trapping *H. ater*, presumably due to the unattractiveness of the compounds trialed. The funnel traps did attract other insects mainly members of the beetle families Scarabaeidae, Cantharidae, Elateridae and Carabidae, as well as cockroaches and termites.

The field trials showed that none of the commercially available compounds enhanced the attractiveness of *P. radiata* billets to *H. ater* adult beetles. The effectiveness of the sachets for their recommended six week longevity could not be compared due to their unattractiveness.

5.5.2.2. Laboratory bioassay of potential volatile compounds

The laboratory bioassay of the volatile compounds did not show any significant difference between the recorded *H. ater* responses. Even though the responses were not significant, the aggregation pheromone MCOL and the antiaggregation pheromone MCH appeared at opposite ends of the range of positive response means. *P. radiata* host material resulted in 100% attraction of *H. ater* adult beetles. The positive response to complete *P. radiata* material and not to individual compounds found in *P. radiata* indicates that the attraction is a result of a complex of compounds and not one individual compound where concentration may also be an important factor.

5.5.2.3. Role of *P. radiata* needles

From this simple experiment with no *H. ater* invasion into the cardboard cylinders, it was concluded that pine needles alone were not involved as an attractant.

5.5.2.4. Role of *P. radiata* bark

H. ater will enter billets of both *Acacia* and *Eucalyptus* spp. wrapped in *P. radiata* bark. Adult *H. ater* beetles entered the bark and slightly engraved the wattle and eucalypt billets, before leaving due to the billets being unsuitable for further gallery construction.

Trial 5.6 was established at a time when *H. ater* should have been present in large numbers, but due to overcast conditions and temperatures below 10°C there was little beetle activity, so the second trial was established. Even though low beetle numbers were present during trial 5.6 invasion was only recorded in the *P. radiata* control billets and the non-host billets wrapped in pine bark.

In trial 5.7 the wattle billets wrapped in pine bark with a lining of paper towel soaked in sugar solution showed a higher number of entry sites compared to the wattle billets just wrapped in pine bark. This could have been due to the sugar solution maintaining the moisture content and suitable conditions similar to the pine control billets which attracted the most *H. ater* adult beetles. However, the sugar solution may have acted as a substrate for microbial growth which in turn may have been responsible for the boosted response.

The attraction of the black pine bark beetle to wattle and eucalypt billets wrapped in *P. radiata* bark demonstrates the specific attractive capacity of the pine bark. Thus, this material must play a significant role in the initial host selection stage.

The attraction of *H. ater* to *P. radiata* material must be in response to a combination of volatile compounds emanating from the host material, and not one individual compound.

5.5.2.5. Role of *P. radiata* chemicals

The absence of *H. ater* beetles on any of the bottle attraction traps and only a low catch on the bark resemblance attraction traps suggests that the traps are not an effective means of trapping the beetles. When the traps were used previously in initial trials they were able to catch some *H. ater*, so the quantity of the solutions would possibly appear too weak to attract beetles when in competition with the surrounding mass of *P. radiata* host material. The experiments were established at times when *H. ater* was present, so beetle numbers should not have been a problem. The difficulty of trialing *P. radiata* host compounds separate from *P. radiata* billets was demonstrated.

5.5.2.6. Role of α-pinene

Adult *H. ater* beetles were attracted to the wattle billets which were coated in α-pinene being captured on the 'Stick-Um'coated billets. It was not possible to detect whether the beetles may have been attracted to the wattle billets coated in α-pinene without the 'Stick-Um', but left again without making any entry into the bark having found it unsuitable as no entry holes on the bark were visible.

The results indicate that the monoterpene, α-pinene must play a role in the attractive ability of *P. radiata* host material, having exhibited in this small experiment the power to attract *H. ater* to a non-host tree by its presence.

5.5.3. Analysis of Feeding Beetles and Frass

The analysis separately of feeding beetles and their host material *P. radiata* over time clearly showed the presence of monoterpenes and monoterpene based compounds and their general fluctuations over time.

The technique is not strictly qualitative but the general trends detected over time were consistent over the treatment and control replicates. The general declines detected for the main monoterpene compounds α-pinene, β-pinene, δ-3-carene, myrcene and terpinolene in both the treatment and control vials may be due to exhaustion of the vials or ventilation. For each of the eight sampling times that the Supelco SPME syringe was inserted into the vials a percentage of the volatiles were collected. The vial lids also had small holes to enable adequate ventilation for the beetles, which would have allowed escape of volatile compounds. But the increase detected for the compounds cymene, fenchone, camphor, isopinocamphone and particularly the unknown compound are distinct real changes. The detection of the $C_9H_{16}0$ unknown compound from the feeding beetle samples and not the control samples was a significant finding. This compound does not match any compounds from the NIST or inhouse CSL databases or any of the compounds isolated from other bark beetles. The unknown compound may be a modified monoterpene or may be produced by the beetles.

GC-MS analysis of *H. ater* frass and the soxhlet extract of accumulated *H. ater* frass showed that the detected monoterpenes, oxidised derivatives and the unknown compound were present in the frass. The unknown compound was present in very low levels in the soxhlet extract, which was of no further assistance with determining the type of compound and identification. Extensive time involved investigations would be required to enable further identification of the unknown compound which was beyond the scope of this project.

H. ater invade host material with a mass attack achieved within seven to ten days under Tasmanian conditions. The oxidised monoterpenes and the unknown compound are produced by feeding *H. ater* beetles after this time interval in quite low levels so would appear to act within the host once the majority of the beetles have arrived. These compounds do not appear to play a role in the initial primary attraction but may act in some way within the host.

5.5.4. Analysis of *P. radiata* Material

Monoterpene hydrocarbons detected in freshly harvested and dry *P. radiata* were generally the same. The main difference in the presence of monoterpenes and monoterpene based compounds detected in fresh and dry *P. radiata* was the oxidised monoterpenes which occur in dry material.

Similar results were noted by Simpson and McQuilkin (1976) who found only minor changes in monoterpene composition of *P. radiata* material with time after harvesting. The same oxidised compounds detected in dry *P. radiata* material including camphor, pinocamphone and isopinocamphone were also detected by Simpson and McQuilkin (1976) over time since harvest. Although it was demonstrated by Hodges and Lorio (1975) that moisture stress can alter monoterpene composition of *Pinus taeda* L. trees.

Water soaking of both fresh and dry material and the freezing treatment resulted in a general decline in the level of compounds but no real differences in the compounds detected. The demonstrated increased attraction to water soaked

billets, was shown by GC-MS to be not due to an increased release of monoterpene hydrocarbons.

Similarly, studies conducted by Ikeda et al (1981) identified no real difference in the composition of monoterpenes present in chemically injured tissue of *Pinus densiflora* Seib. & Zucc. compared to uninjured tissue.

From the results of this study, it is assumed that the freezing of *P. radiata* material, has little impact on its monoterpene composition. The beneficial effect of damaged material for *H. ater*, would be the provision of suitable breeding sites due to the rupturing of cells.

CHAPTER 6

HOST SELECTION AND ATTRACTION - MICROBIAL ASPECTS

6.1. INTRODUCTION

This investigation was initiated to study the microorganisms associated with *H. ater* and their possible role in host selection and colonisation. Within the Scolytidae many associations exist between beetles and microorganisms which contribute to their success (Webb 1945, Francke-Grosmann 1963, Whitney 1971, Barras & Perry 1972, French & Roeper 1972, Brand et al 1975, 1977, Brand & Barras 1977, French et al 1982, Bridges 1983, Wingfield & Swart 1989). Whitney (1982) details approximately one hundred different microorganisms which have been identified as being associated with bark beetles. The microorganisms include fungi, bacteria, yeasts and protozoans which play a role in location of hosts, adult and larval nutrition and establishing conditions favourable for beetle development (Whitney 1982, Beaver 1989).

H. ater has defied what is known with regard to other economic species of Scolytidae that have been examined. Using the standard procedures employed in scolytid research, repeated testing, and the chemical expertise of Dr N. Davies of the CSL (University of Tasmania) no major volatiles were isolated from adult *H. ater* beetles using whole beetle extraction and headspace techniques other than host tree compounds and derivatives. Trials of compounds which have been reported as sex attractants or repellents with primary bark beetles as well as host volatile monoterpenes implicated that host volatile monoterpenes particulary α-pinene as well as possibly MCOL are involved in *H. ater* host selection and colonisation. The attraction of *H. ater* following the rewetting and burial of dried out *P. radiata* billets which still retain bark, but not to similarly treated non host material strongly suggests a species specific residual character most likely affected by microorganisms.

There must be some other factor involved in beetle attraction in combination with the beetles and host. Several workers (Hetrick 1949, Franck-

Grosmann 1963, Graham 1967, Graham 1968, Barras 1969, Barras 1970, Cade et al 1970, Moeck 1971, Whitney 1971, Berryman 1972, Barras 1973, Brand et al 1977, 1979, Moeck 1981, French et al 1982, 1984, Raffa & Berryman 1982, Bridges et al 1985, Owen et al 1987, Lieuter et al 1989) have found that the microbial-bark beetle associations influence the host selection and colonisation stages and brood development.

This led to the hypothesis that the host selection and attraction process could involve the beetles plus the host responding to the action of microorganisms namely yeasts, bacteria and fungi.

This firstly involved the isolation of bacteria, yeasts, and fungi associated with *H. ater* and *P. radiata* host material and their subsequent preliminary identification. In laboratory based trials the response of *H. ater* to the bacterial, yeast and fungal isolates was then recorded. The bacterial and yeast beetle isolates were also trialed under field conditions. From the response of *H. ater* the role of individual bacterial, yeast and fungal species in host selection and colonisation was evaluated. The attractiveness of *P. radiata* billets which were excluded from contact with normal microorganisms and the attractiveness of damaged *P. radiata* billets was also evaluated. However an extensive study of the micrororganisms associated with *P. radiata* billets was not conducted.

For many bark beetles fungal organisms are carried in mycangial pits on the surface of the body (Batra 1963). The aim was to determine whether the commonly associated fungus *Leptographium lundbergii* Lagerberg & Melin and other fungal isolates are carried in mycangia or otherwise. Adult *H. ater* beetles were examined for such structures and for fungal spores using SEM.

A simple experiment was performed which involved the addition of autoclaved and nonautoclaved *H. ater* beetles to the 3% malt extract agar (MEA) used to culture *L. lundbergii*, to determine if the presence of *H. ater*, influenced the growth of the fungus in any way.

Pathogenic fungi play an important role in the control of insect pests. The pathogenic fungi *Metarhizium anisopliae* (Metsch.) Sorok. and *Beauveria*

bassiana Bals. Vuill. occur in several species of Scolytidae (Whitney et al 1984) and were briefly tested against *H. ater* under laboratory conditions to see if they could be a potential direct control method against *H. ater*.

6.2. LITERATURE REVIEW

6.2.1. General

The involvement of microorganisms in the host selection process of bark beetles was first suggested by Person (1931). As Person wrote in 1931 "It was also found during 1928 that fermenting inner bark was more attractive than any of the other substances tested. This suggested the possibility that the attractiveness of attacked trees might be due to some fermentation organism, such as a yeast, associated with the beetle. Following the successful initial attack by a few pioneer beetles, a second, stronger attraction is exhibited as a result of the yeast introduced via the attacking beetles" (Person 1931).

6.2.2. Fungal Association

6.2.2.1. Bark beetle-fungal associations

Numerous beetle-fungus associations exist within the Scolytidae. These relationships have been acknowledged for a long time (Leach et al 1934). Many bark beetles which attack conifers carry spores of various fungi, especially from the genera *Ceratocystis* Ell. & Halst. and *Ophiostoma* H. & P. Syd. (Francke-Grosmann 1963, Berryman 1989). Whitney (1982) lists 21 species of *Ceratocystis* in association with bark beetles in North America.

The southern pine beetle, *D. frontalis*, has an association with three fungal species (Coppedge et al 1995). Ascospores of *Ophiostoma minus* Hedgecock (= *Ceratocystis minor* Hedgecock) are carried externally, and *Ceratocystiopsis ranaculosus* Upadhyay & Kendrick and an unidentified basidiomycete occur in prothoracic mycangia of female beetles (Bridges 1983, Coppedge et al 1995).

The ambrosia beetle, *Xyleborus dispar* Fabricius has a symbiotic relationship with the fungus *Ambrosiella hartigii* Batra (Batra 1967).

The fungi *Ceratocystis montia* Rumb. and *Europhium clavigerum* Parker, are closely associated with the mountain pine beetle, *D. ponderosae* (Whitney 1971). *D. ponderosae* also transmits *Ophiostoma ips* Ruhm (= *Ceratocystis ips* Ruhm) and *Ceratocystis clavigera* Robins.-Jeff. & Davids. Updhyay (Owen et al 1987).

The western pine beetle, is associated with *C. minor* and *Ceratocystis nigrocarpa* Davids. and an unidentified basidiomycete (Owen et al 1987).

The red turpentine beetle, *D. valens*, transmits *C. ips*, *Leptographium terebrantis* Barras & Perry, and a *Graphilbum* spp. (Barras & Perry 1972).

The Australian ambrosia beetle, *Platypus subgranosus* Schedl, is a vector of the fungus, *L. lundbergii* (Webb 1945).

The six fungi *O. ips*, *Leptographium serpens* Goid., *Leptographium truncatum* Wingfield & Marasas, and unidentified species of *Ophiostoma*, *Leptographium*, and *Graphium* were isolated from *Hylastes angustatus* Herbst, and *H. ligniperda* (Wingfield & Swart 1989). *L. serpens* and an unidentified *Leptographium* spp. were isolated from *H. ater* adult beetles in England (Wingfield & Gibbs 1991). *L. truncatum* is associated with *H. ater* and *H. ligniperda* in New Zealand (Wingfield et al 1988). The synonomy of *L. lundbergii* and *L. truncatum* is suggested by Wingfield and Gibbs (1991).

H. nigrinus vectors the fungus *Verticicladiella wageneri* Kendrick (*Ceratocystis wageneri* Goheen & Cobb) (Witcosky et al 1986).

The mutualistic fungus *Trichosporium symbioticum* Wright has been isolated from the fir engraver beetle, *S. ventralis* (Wright et al 1979).

6.2.2.2. Production of volatile compounds

Many bark beetle pheromones are derived from α-pinene and microorganisms may play a role in the transformation (Brand et al 1979).

Badcock (1939) describes the numerous odours produced by wood destroying fungi. Numerous metabolic volatiles have been isolated from wood rotting fungi. The methyl esters of p-methoxycinnamic, cinnamic, and anisic acids, have been isolated from the fungus *Lentinus lepideus* Fr, and methyl anisate and anisaldehyde from the fungus *Trametes suaveolens* Fr. (Birkinshaw & Morgan 1950).

Endoconidiophora coerulescens Mu'nch produces isobutyl acetate, methyl heptenone (2-methylhept-2-en-6-one) and a mixture of methyl heptenols (*l*- and *dl*- 2-methylhept-2-en-6-ol), and *Endoconidiophora virescens* Mu'nch produces methyl heptenone and a mixture of *l*- and *dl*- methylheptenol (Birkinshaw & Morgan 1950). These compounds are responsible for the characteristically strong odour of the two *Endoconidiophora* species (Birkinshaw & Morgan 1950).

Collins and Halim (1970) describe the production of monoterpenes by the filamentous fungus *Ceratocystis variospora* (Davidson) C. Moreau. The odorous constituents of *Trametes odorata* Fr. have been identified as phenylacetate, citronellol, nerol, geraniol and methyl p-methoxyphenylacetate (Halim & Collins 1971).

Brand et al (1976) noted that fungi present in the mycangia of *D. frontalis* beetles were able to convert *trans*-verbenol to verbenone. Brand and Barras (1977) detail work with a basidiomycete isolated from female southern pine, *D. frontalis* beetles which has a characteristic fruity odour in culture, and the odour is also distinct in the phloem of pine trees infested with the beetles. The major volatiles produced by the fungus were identified as isoamyl alcohol, 6-methyl-5-hepten-2-one, and sulcatol.

The fungus *Aspergillus niger* van Tiegh. was shown to convert *dl*-α-pinene into *cis*-verbenol and verbenone (Bhattacharyya et al 1960, Prema & Bhattacharyya 1962).

Collins (1976) describes the isolation of numerous volatiles from a range of fungal species including *Streptomyces* spp., *Trichoderma viride* Pers., *Penicillium decumbens* Thom., *Phellinus* spp., *Ceratocystis* spp. and *T. odorata*.

6.2.2.3. Role of beetle-fungal associations in host selection and colonisation

The role of the beetle-fungal associations have been shown to influence the host selection and colonisation stages and brood development, often in overcoming host resistance (Cook & Hain 1987, Owen et al 1987, Raffa & Smalley 1988, Lieutier et al 1989).

The response of *P. pinaster* and *P. sylvestris* to attack by the two bark beetles *T. piniperda* and *Ips sexdentatus* Boerner and their associated fungi (respectively *Leptographium wingfieldii* Morelet and *Ophiostoma brunneo-ciliatum* Math.- K. Hunt), results in a considerable increase in the concentration of all the phloem monoterpenes (Lieutier et al 1989).

Work by Raffa and Berryman (1982) details the accumulation of monoterpenes and other volatiles as a result of the inoculation of grand fir with a fungus transmitted by the fir engraver, *S. ventralis*.

An increase in the level of terpenes in sapwood of lodgepole pine was observed following the attack by *D. ponderosae* and associated microorganisms (Shrimpton 1973). The fungi *C. montia* and *E. clavigerum* associated with *D. ponderosae* are implicated in overcoming host resistance (Webber & Gibbs 1989).

Ips cembrae Heer in association with *Ceratocystis laricicola* (Bliss) C. Moreau, resulted in the dieback and death of larches in England (Redfern 1989).

The fungi associated with *D. frontalis* are able to convert *trans*-verbenol (an aggregation pheromone) to verbenone (an antiaggregation pheromone) (Brand et al 1976, Brand & Barras 1977). The fungi are also capable of synthesising sulcatol, which is a pheromone of the ambrosia beetle *G. sulcatus* (Brand et al 1976, Brand & Barras 1977).

Work conducted by Barras (1973) showed that the removal of the mycangial and ectodermal fungi associated with the southern pine beetle, *D. frontalis* resulted in a significant decrease in the number of progeny, progeny/gallery, progeny/cm of gallery, and delayed initial emergence. No effect was observed on the number of successful attacks, length of ovipositional gallery, and number of egg niches/cm of gallery (Barras 1973). Coppedge et al (1995)

detail the importance of the fungal associates of *D. frontalis* but their presence may influence beetle size and lipid content.

Brand et al (1979) describes the invasion of *S. multistriatus* into the bark which is infected with *C. ulmi*.

It has been suggested that fungal organisms assist in creating a suitable habitat for brood development, by reducing the moisture content of host material (Reid 1961, Mathre 1964, Cook & Hain 1986)

6.2.2.4. Additional roles of beetle-fungal associations

Many researchers have believed that a mutualistic relationship exists between bark beetles and their associated fungi, with the fungi assisting in the establishment of the beetles and in return the fungi being disseminated by the beetles (Francke-Grosmann 1963, Graham 1967, Berryman 1972, Whitney 1982, Webber & Gibbs 1989). Mutualism implies a benefit to both partners in the association (Beaver 1989). The fungi benefit by being transported to a new habitat, with the beetles tunneling activity assisting mycelium growth (Beaver 1989). The benefits to the beetle may include provision of food for adults and larvae; location of hosts; transformation of host tree compounds to beetle pheromones; conditioning the host as a suitable breeding site; and reducing host defence mechanisms (Kok & Norris 1972, Kingsolver & Norris 1977, Whitney 1982, Beaver 1989, Christiansen & Bakke 1989).

Another hypothesis is that the fungi carried together by bark beetles may act in an antagonistic manner to inhibit the effect of primary pathogenic fungi (Hetrick 1949, Barras 1970, Whitney 1971, Bridges & Perry 1985). Antagonistic effects of fungi carried together by bark beetles have been shown by Barras (1969), Bridges and Perry (1985) and Owen et al (1987).

6.2.2.5. Fungal transport structures

Many bark beetles possess specialised structures for carrying their symbiotic fungi, often known as mycangia (Batra 1963). As described by

Berryman (1989), mycangia occur in many places on the insect body as pits, sacs, pouches or tubes formed by invaginations of the cuticle.

The ambrosia beetle, *Platypus wilsoni* Swaine possesses fungal pits or mycangia on the pronotum of female beetles (Farris & Funk 1965). Fungal transport structures consisting of 'small cup shaped pits' are present on the top and sides of the head in the fir engraver, *S. ventralis* (Livingston & Berryman 1972).

Mycangia were described in the bark beetles *H. palliatus*, *H. ater*, and *H. cunicularius*, as cup like punctures of the integument, particularly the elytra, which act as transmission organs for fungi. The mycangia are present in both sexes containing yeast cells and blue-stain fungal spores (Francke-Grosmann 1963).

Female southern pine (*D. frontalis*) beetles, possess two fungi and two yeasts in prothoracic mycangia (Brand & Barras 1977).

The male ambrosia beetles, *G. retusus* and *G. sulcatus* exhibit an enlargement of the forecoxal cavities which contain fungal spores.

Work by Paine and Birch (1983) showed that the symbiotic fungi of *D. brevicomis* is acquired in the mycangia from the fungi lining the pupal chambers.

6.2.2.6. Entomopathogenic fungi

Most of the entomopathogenic fungi contain hyphae (fungal strands developing from a germinating spore) which together constitute a mycelium. Reproduction is mainly by spores which may arise sexually or asexually. Sexual reproduction involves the union of nuclei belonging to two sex cells or gametes. Asexual reproduction generally results in spores born in sporangia (sporangiospores) or on hyphae (conidia).

Most of the entomopathogenic fungi belong to the subdivisions Mastigomycotina, Ascomycotina, and Deuteromycotina (Fungi Imperfecti), and Basidiomycotina (Poinar & Thomas 1978).

Most entomopathogenic fungi initiate infection by a germinating spore which penetrates the cuticle of an insect. The invasive hypha enters the host tissues and spreads through the hemocoel. After filling the dying or dead insect with mycelium, emergence hyphae grow out through the insects integument and produce spores on the external surface of the host. These spores are dispersed by wind or rain and even by the diseased insect during feeding or mating (Poinar & Thomas 1978).

Successful fungal infections depend on a large host population and ideal climatic conditions including adequate moisture and temperature (Poinar & Thomas 1978).

There are several ways pathogenic fungi can injure or kill insects. They include: mycotoxin production; pathological changes in the hemolymph; histolytic action; mechanical blockage of the gut by vegetative growth; and physical damage by mycelial development (Bell 1974).

In the early stages of infection, the insect may show general ill effects such as a cessation of feeding, weakness, and disorientation. The insect often changes colour and the cuticle may show dark spots indicating areas of fungal penetration. Eventually the parasitised insect becomes moribund and soon after death is filled or covered with sporulating hyphae (Poinar & Thomas 1978).

The two pathogenic fungi, *B. bassiana* and *M. anisopliae* belong to the class Fungi Imperfecti (Bell 1974). *B. bassiana* and *M. anisopliae* exhibit a wide host range (Poinar & Thomas 1978). *B. bassiana* and *M. anisopliae* are characterised by powdery white spores and green spores respectively (Poinar & Thomas 1978).

B. bassiana and *M. anisopliae* occur in several species of Scolytidae (Whitney et al 1984). *B. bassiana* has been found to be associated with *S. multistriatus* (Doane 1959 cited in Bell 1974) and *D. frontalis* (Bell 1974). *M. anisopliae* has been found to be associated with the black turpentine beetle, *Dendroctonus terebrans* Olivier (Holt 1961 cited in Bell 1974).

Dryocoetes confusus Swaine infected with *B. bassiana* resulted in the death of adult beetles following egg gallery establishment (Whitney et al 1984).

Milner and Prior (1994) detail the susceptibility of the two most important acridid pests, the Australian plague locust, *Chortoicetes terminifera* Walker, and the wingless grasshopper, *Phaularidium vittatum* Sjostedt to the pathogenic fungi *Metarhizium* spp. The use of *Metarhizium* spp. has the potential of being an effective 'environmentally friendly' control method, compared to the use of the traditional chemical control (Milner & Prior 1994). Application of an oil based spray of *Metarhizium flavoviride* Gams & Rozsypal was effective in controlling the wingless grasshopper, *P. vittatum* (Milner et al 1994).

Investigations with the two fungal pathogens *B. bassiana* and *M. anisopliae*, determined that these fungi required temperatures between 15°C-35°C for spore germination, hyphal development, and spore production. The optimal temperatures for this series of developmental stages in the fungi were between 25° C-30°C. The spores of both fungi were killed near 50°C, and lost their viability after a few months at 21°C, but when stored at 8°C they remained viable for a year (Walstad et al 1970 cited in Bell 1974).

Soils with a high organic content are generally more conducive to fungal infections than are light sandy soils which lack the water retention properties of organic matter (Bunzli & Buttiker 1959 cited in Bell 1974). They also found that soils with very high amounts of nitrogenous organic matter favoured development of certain insects and also provided the proper conditions for growth, development, and high virulence in the fungal pathogens *B. bassiana* and *M. anisopliae*. Soil pH also influences fungal activity.

6.2.3. Yeast Association

Bark beetles attacking coniferous trees in North America are associated with 18 species of yeasts (Whitney 1982). Yeasts are believed to be involved in the nutrition of the beetles, and in return are disseminated by the beetles (Walt

1972) although the relationship between yeast and bark beetles is not fully understood (Phaff & Starmer 1987).

Scolytid beetles have been shown to be attracted to ethanolic odours (Graham 1968, Cade et al 1970, Moeck 1971) produced by microorganisms including yeasts (Soumalainen 1969, Brand et al 1977). The by-product of yeast and fungal metabolism, ethyl alcohol, is attractive to scolytid beetles (Moeck 1981, Elliott et al 1983), with many bark beetles being attracted to stressed or dying trees with fungal and bacterial infections (Beaver 1989). Ethanol is produced by ageing log tissues and can be produced by living tree tissues under anaerobic conditions (Cade et al 1970, Kelsey 1994).

Moeck (1981) details work where ethanol induced attack on spruce trees, *P. engelmanni*, by spruce beetles, *D. rufipennis*.

Anaerobic conditions induce attack by the striped ambrosia beetle, *T. lineatum*. Kelsey (1994) describes the colonisation by the ambrosia beetle, *T. lineatum* of Douglas fir logs without branches compared to logs with branches. The concentration of ethanol, acetaldehyde and water was higher in the delimbed logs (Kelsey 1994).

Yeasts in particular *Candida nitratophila* Shifrine & Phaff Meyer present in the gut of *I. typographus* are capable of interconverting *trans*- and *cis*-verbenols to verbenone (Leufv'en et al 1984).

The yeasts *Pichia pinus* (Holst) Phaff, *Hansenula capsulata* Wickerham, and *Hansenula holstii* Wickerham are closely associated with *D. ponderosae* (Whitney 1971, Hunt & Borden 1990). *P. pinus* and *H. capsulata* associated with *D. ponderosae* are able to convert *cis*- and *trans*-verbenol to verbenone (Hunt & Borden 1990).

The yeasts *H. holstii*, *P. pinus*, and *Pichia bovis* van Uden et have been isolated from the Southern pine beetle, *D. frontalis* (Brand et al 1977), with both *H. holstii* and *P. pinus* being found in the mycangium of female beetles (Barras & Perry 1972). *H. holstii* and *P. pinus* produce ethanol, isoamyl alcohol, and 2-

phenyl ethanol, and *P. bovis* produces isoamyl acetate and 2-phenylethyl acetate (Brand et al 1977).

Brand et al (1977) showed that volatile metabolites produced by the yeasts associated with the Southern pine beetle, are able to enhance the attractiveness of a mixture of frontalin, *trans*-verbenol and turpentine in laboratory bioassays.

6.2.4. Bacterial Association

Whitney (1982) lists 18 species of bacteria associated with North American bark beetles. Numerous workers have implicated bacterial species in playing a role in the attraction and host selection process of a number of Scolytidae.

Bacillus cereus Frankland & Frankland isolated from the gut of adult male and female *I. paraconfusus* beetles was capable of transforming α-pinene to *trans*- and *cis*-verbenol, as well as *trans*-pinocarveol and myrtenol (Brand et al 1975).

A *Bacillus* spp. has been isolated from both male and female *I. grandicollis* and three species of *Dendroctonus* that is capable of converting α-pinene to both verbenols (Brand et al 1975). This indicates that microorganisms could play a role in the production of pheromones within bark beetle frass (Brand et al 1975).

The potential role of bacteria in host attraction has been demonstrated for members of Scolytidae. French (1972) [in French et al 1982] observed that *X. dispar* attacked host material infected with *Pseudomonas syringae* van Hall and suggested "that volatiles given off by bacterial metabolism were a primary cause of attraction for pioneer *X. dispar*". French et al (1982) found that gamma irradiating elm billets, *Ulmus procera* Salisb., reduced their attractancy to the smaller elm bark beetle, *S. multistriatus*. This may have been due to the removal of microorganisms or a change in plant volatiles or a combination of these factors (French et al 1982).

French et al (1984) examined the response of *S. multistriatus* to several bacterial species isolated from host material. The bacterial isolates included

Bacillus subtilis Ehrenberg Cohn, *Bacillus pumilus* Meyer & Gottheil, and *Enterobacter cloacae* Jordan Hormaeche & Edwards.

Tudroszen et al (1977) detail work on the characterisation of intermediates in α-pinene metabolism by *Pseudomonas putida* (Trevisan) Migula, as 3-isopropylbut-3-enoic acid and (Z)-2-methyl-5-isopropylhexa-2,5-dienoic acid.

All work concerning the production of beetle pheromones by microorganisms has been performed in the laboratory, and little is known of the role of fungi and bacteria in the production of pheromones in the field (Berryman 1989).

6.3. MATERIALS AND METHODS

6.3.1. Bacterial and Yeast Associations

6.3.1.1. Isolations and identification

Two 17 year old *P. radiata* trees were felled and cut into 40 billets of 40 cm length. The billets were buried horizontally 2/3 in the soil at seven sites throughout the study site. The billets were used to source *H. ater* adult beetles for bacterial and yeast isolations.

Bacteria and yeasts were isolated from 40 *H. ater* adult beetles. Individual beetles were briefly placed in 75% alcohol, transfered to a 10% sodium hypochlorite solution for 15 min, followed by washing in sterile water. Dissection of the alimentary tract was performed in sterile Ringers solution (9 g NaCl, 0.4 g KCl, 0.4 g $CaCl_2$, 0.2 g $NaHCO_3$, 1000 ml water). The gut was macerated with a glass rod in 9 ml 0.85% NaCl and a serial dilution 10^{-2}-10^{-8} prepared in 0.85% NaCl. A 0.1 ml aliquot was plated onto Potato dextrose agar (PDA) for fungal isolation and Tryptone soy agar (TSA) for bacterial and yeast isolation, with two plates/dilution/media. One set of plates was incubated aerobically and one anaerobically at 25°C. The dominant biota was then recultured onto the respective media to check for purity. Isolations were also made from 40 individual non sterile adult beetles following individual maceration in 9 ml 0.85% NaCl and

preparation of serial dilutions as previously explained. Bacteria and yeasts were also isolated from over 100 *H. ater* adult beetles allowed to walk over the agar plates. Bacterial and yeast isolates were maintained on nutrient agar slopes.

The standard preliminary biochemical identification tests were conducted with the isolates including Gram stain, determination of motility, oxidative/fermentative utilisation of glucose and growth on cellulose, starch and xylan. The Imvic tests for Enterobacteria were also performed including Kosers citrate broth for citrate utilisation, Glucose peptone broth for Voges-Proskauer test and methyl red test, and Tryptone water for indole production.

Additional *P. radiata* billets of 40 cm length were completely enclosed in wire mesh and buried vertically 2/3 in the soil. The aim was to prevent beetles from entering billets by covering the billets in mesh so they contacted the soil and normal microbiota but no microorganisms could be introduced to the billet from the beetles. The first trial involved the use of the smallest diameter flywire mesh available wrapped individually around three *P. radiata* billets which were buried with three control *P. radiata* billets. The second trial involved the use of the smallest diameter brass mesh individually enclosing two *P. radiata* billets with two control *P. radiata* billets buried at the same time. A third trial involved the burial of three *P. radiata* billets in field soil in plastic containers in the laboratory in isolation from *H. ater* beetles.

6.3.1.2. Bacterial/Yeast laboratory trials

The ten bacteria and two yeasts isolated from *H. ater* were trialed for attractiveness in the laboratory using a Y-tube olfactometer. The Y-tube was connected to a small pump drawing air through the tubes at a flow rate of 100 ml/min (as described in Chapter Five).

The isolates were grown in nutrient broth, nutrient broth plus 10 mm disc of phloem and bark, phloem broth consisting of nutrient broth plus phloem filtrate, and sugar ferment solution. A 10 mm disc of filter paper was dipped in each culture and placed at the end of one of the Y-tube arms. Twenty beetles were

tested for each isolate and medium, with the test solution placed on one side of the Y-tube and then the other. Individual adult beetles were placed in the Y-tube having the choice of moving forward in the left or right arm of the Y-tube, moving backwards or not moving.

6.3.1.3. Bacterial/Yeast field trials

6.3.1.3.1. Bacterial/Yeast field trial 6.1

Three replicates of 19 billets were buried vertically (2/3 below the soil surface) arranged in a circle configuration of six metres in diameter with each billet separated by approximately one metre. The 20 cm long billets were cut from the same freshly harvested *P. radiata* tree.

Glass McCartney bottles containing media and bacterial and yeast isolates were attached to each billet with strong tape (vertically aligned with the billet), and the metal screw cap lids had a 1 mm hole in the top to enable the release of odours. The isolates were selected at random from the twelve bacteria and yeast cultures isolated from *H. ater* being isolate numbers 1, 2, 3, 8 and 11. The isolates were grown in three media consisting of nutrient agar, nutrient broth plus additional disc of *P. radiata* phloem and bark, and phloem broth consisting of nutrient broth plus additional phloem filtrate. The bottles of isolates were inoculated and incubated at 25°C for five days prior to field establishment.

Each replicate contained one of each of the following treatments:

Control billet

Billet + uninoculated nutrient broth

Billet + uninoculated nutrient broth plus disc of phloem and bark

Billet + uninoculated phloem broth

Billet + inoculated nutrient agar x 5 isolates

Billet + inoculated nutrient broth plus disc of phloem and bark x 5 isolates

Billet + inoculated phloem broth x 5 isolates

The trial was established for five weeks, with the number of entry holes recorded each week, and at the completion of the trial the bark was removed from each billet and the number of adult beetles present recorded.

6.3.1.3.2. Bacterial/Yeast field trial 6.2

As a result of laboratory bioassays the bacterial and yeast isolate numbers 1, 5, 6, 7 and 9 were selected to investigate their attractiveness in the field utilising bottle traps. A sugar ferment solution was prepared as a medium for the bacterial and yeast isolates (133.3 g sucrose, 6.6 g KNO_3, 2.0 g $MgSO_4$, 2.0 g yeast extract and 1000 ml water). The bottles of media were inoculated with the isolates and incubated at 25°C for six days prior to field establishment.

At each of three sites the following treatments were replicated once at each site.

Control sugar ferment solution

Sugar ferment solution + Isolate 1

Sugar ferment solution + Isolate 5

Sugar ferment solution + Isolate 6

Sugar ferment solution + Isolate 7

Sugar ferment solution + Isolate 9

The following establishment method was employed. Wooden stakes were placed in the ground and the bottles containing the respective treatments were placed at the base of the stakes and a cylinder of mesh coated in 'Stick-Um' placed over the stake keeping the bottle in place. A wick made of cotton was placed in each bottle so that it extended up to the top of the mesh cylinder. The traps were established for a period of five weeks, being checked on a weekly basis with any beetles present collected.

6.3.1.3.3. Bacterial/Yeast field trial 6.3

Another trial was established using the same procedure as outlined for trial 6.1. The exceptions were the use of isolate numbers 1, 5, 6, 7 and 9 and the

incubation of the isolates at 25°C for six days prior to field establishment. This trial was established for three weeks, being checked weekly, with the number of entry holes recorded. At the completion of the trial the bark was removed from each billet and the number of beetles present recorded.

6.3.1.3.4. Inoculated billets

Thirty-five billets 35 cm in length were cut from a freshly harvested *P. radiata* tree. The billets were endcoated with mastic to prevent moisture loss and stored at 4°C. Isolates 1, 5, 6, 7 and 9 were cultured in nutrient broth and incubated for four days at 25°C.

The billets were inoculated with 0.3 ml of the various isolate and nutrient broth solutions at 16 sites (four sites in each quarter of the billet). Each inoculation site was prepared by removing a core of bark and phloem with a sterile cork borer 1 cm in diameter. Each inoculation site was packed with sterile cotton wool plugs following inoculation and secured with tape. The billets were incubated at 25°C for two days and then taken into the field.

From each end of the billet 2.5 cm was removed creating a fresh surface without the mastic and a billet of 30 cm in length. The billets were buried vertically in the soil with 1/4 of the billet above the soil surface in a circle configuration. Five replicates each containing one of the following treatments were established

Billet inoculated with Isolate 1

Billet inoculated with Isolate 5

Billet inoculated with Isolate 6

Billet inoculated with Isolate 7

Billet inoculated with Isolate 9

Control billet inoculated with nutrient broth

Control uninoculated billet

The trial was established for a period of four weeks, checked weekly for entry holes and when dismantled the bark was removed from each billet and number of beetles recorded.

6.3.1.3.5. Sterilisation trials

Two trials were established to compare *H. ater* invasion of control *P. radiata* billets with billets which were sterilised being buried in sterile sand to determine if removal of any microflora associated with *P. radiata* and eliminating contact with soil microflora influenced the attractiveness of *P. radiata* material.

6.3.1.3.5.1. Sterilisation trial 1

Six billets one metre in length were cut from a freshly harvested tree, the ends sealed with mastic to prevent moisture loss and stored at 4°C. Within 24 h of harvest three small billets 30 cm in length were cut from each long billet and one of each used for the three treatments of control, sterilised and surface sterilised. For the sterilisation treatment the billets were individually wrapped in brown paper and autoclaved for 30 min. The surface sterilisation treatment involved the billets being dipped into boiling water, removed and placed in sterile plastic bags. Within 24 h of treatment the billets were established individually under field conditions being buried vertically in sterile sand within a sterile heavy duty plastic bag which was placed in a hole level with the soil surface. The bags had a drainage tube attached to the base to allow water to drain. Three replicates of each treatment were buried at two different sites as three rows of three billets.

The billets were checked seven times over the duration of 64 days that the trial was established, with the number of entry holes recorded. At the completion of the trial the bark was removed from all billets and the number of beetles recorded.

6.3.1.3.5.2. Sterilisation trial 2

The second trial utilised the same method as described for the first trial except some different treatments were involved. The following treatments were replicated once at three sites.

Sterilised billet in sterile sand in bag

Frozen billet in sterile sand in bag

Control billet in sterile sand in bag

Sterilised billet in field soil in bag

Frozen billet in field soil in bag

Control billet in field soil in bag

Control billet in field soil with no bag

The billets were checked on a weekly basis for the 28 days that the trial was established, with the number of entry holes recorded each week. At the completion of the trial the bark was removed from all billets and the number of beetles recorded.

6.3.1.3.6. Attraction to damaged *P. radiata*

A trial was established to compare the attractiveness of *P. radiata* billets which had been damaged by subjecting to a freezing treatment Three billets one metre in length were cut from a freshly harvested tree, endcoated with mastic to prevent moisture loss and placed in a -20°C freezer for one week. Following removal from the freezer two smaller billets 40 cm in length were cut from each billet. The six billets were established under field conditions in combination with six more billets of 40 cm length cut from a freshly harvested tree. At two sites three frozen and three fresh billets were buried vertically with 2/3 below the soil surface, in a circle configuration of approximately three metres in diameter.

The billets were checked seven times over the duration of 64 days that the experiment was established, with the number of entry holes recorded. The trial was then dismantled with the bark being removed from the billets and the number of beetles present recorded.

6.3.2. Fungal Associations

6.3.2.1. Scanning electron microscopy

The adult *H. ater* beetles used for SEM were collected just prior to their preparation as described. The adult beetles were killed and left in a desiccator for 12 h, and then mounted on the specialised stubs with double sided sticky tape. Some beetles were mounted untreated, while others were fixed with osmium tetroxide vapour from dry crystals to enable observation of the fungus under the scanning electron microscope. Five male and five female adult *H. ater* beetles were studied with dorsal, lateral and ventral views, as well as dorsal and lateral views of the elytra.

6.3.2.2. Fungal isolations and laboratory olfactometer trials

Fungi were isolated internally and externally from *H. ater* adult beetles using the same method and same beetle source as detailed for bacteria and yeast isolations.

Fungal isolates were also cultured directly from over 100 live adult *H. ater* beetles allowed to walk on MEA and Sabourauds dextrose agar (SAB). The fungi were incubated at 25°C and room temperature. Fungi were also isolated from the galleries and tunnels of *H. ater* by transferring spore masses directly to MEA.

Fungal isolates which were associated with bacteria were separated by culturing on either 2% MEA with 0.1% streptomycin and 100 ppm cycloheximide or 1% MEA and 50 mg/l penicillin, 50 mg/l streptomycin and 25 mg/l polymixin.

The most commonly associated fungi isolated from *H. ater* were tested in the laboratory using a Y-tube olfactometer as described for the bacterial and yeast olfactometer trials. Discs 10 mm in diameter of each culture were removed from the petri dish at various times during the duration of fungal growth and tested using the Y-tube apparatus.

6.3.2.3. Influence of *H. ater* on the growth of *Leptographium lundbergii*

To determine if *H. ater* influenced the growth of *L. lundbergii* (the most commonly associated fungal species) autoclaved and nonautoclaved adult beetles were added to the agar used to culture the fungus. The control treatment consisted of 3% MEA, the autoclaved treatment consisted of 3% MEA with the addition of autoclaved crushed beetles, and the nonautoclaved treatment consisted of 3% MEA with the addition of nonautoclaved crushed beetles. Fifteen plates of each agar treatment were inoculated with *L. lundbergii* culture and incubated at room temperature. The cultures were examined at regular intervals and the rate of expansion recorded.

6.3.2.4. Entomopathogenic fungal trials

Conidia of *M. anisopliae* isolate FI522 were obtained from the CSIRO Insect Pathogen Culture Collection of the Division of Entomology, Canberra. The *B. bassiana* culture was obtained from the CRC for Temperate Hardwood Forestry, Division of Resource Protection, Tasmania, which had been isolated from Chrysomelid beetles. The cultures of *M. anisopliae* and *B. bassiana* were maintained on SAB (65 g SAB, 4 g yeast, 1000 ml water) at 25°C.

Two trials in the laboratory were established with both cultures being tested against *H. ater* using the following method. Adult beetles were collected from *P. radiata* billets just prior to treatment exposure. The trials were conducted over the summer period.

Treatment A involved allowing the adult beetles to just walk over the fungal surface for two minutes. Treatment B involved deliberately covering the adult beetles in fungal spores. Ten adult beetles were used for each treatment at the two temperatures of 10°C and 20°C.

Following exposure to the fungal spores the adult beetles were placed in plastic containers of 10 cm diameter, with air holes in the top and a moistened filter paper on the bottom and a sterilised piece of bark. The containers were monitored at regular intervals and the moisture maintained.

A third trial was established applying *M. anisopliae* directly and in peanut oil to *P. radiata* bark to which adult *H. ater* beetles were added. The number of *M. anisopliae* spores in the peanut oil was counted to be 3.8 x 10^7/ml using a Petroff-Hausser counting chamber.

The recording of beetle deaths for all trials was timed accordingly at 10, 17 and 38 days after establishment, due to concurrent field work over the summer period.

6.4. RESULTS

6.4.1. Bacterial and Yeast Associations

6.4.1.1. Isolations and identifications

Bacteria and yeasts were only isolated from agar plates incubated aerobically at 25°C with no growth present on agar plates incubated anaerobically. Ten bacterial and two yeast isolates were found in constant association with adult *H. ater* beetles as shown in Table 6.1. From the basic identification tests the bacterial isolates were identified as belonging to the family Enterobacteriaceae, and provisionally the genera Edwardsiella, Klebsiella, Citrobacter and Pseudomonas/Alcaligenes.

It was not possible to isolate microorganisms from *P. radiata* billets covered in mesh to exclude *H. ater* and buried under field conditions due to *H. ater* beetles being able to enter the billets through the small holes in both types of mesh which were trialed. Slightly larger *H. ater* adult beetles were visible with their bodies half way through the holes in the mesh.

None of the usual microflora was found on *P. radiata* billets buried in the same soil but in isolation from *H. ater* in the laboratory, that was commonly associated with *H. ater*. No similar microorganisms were isolated from *P. radiata* material and *H. ater* beetles, so only the microorganisms associated with *H. ater* were used in laboratory and field bioassays. To determine if beetle associated microrganisms are involved in host selection and attraction.

6.4.1.2. Bacterial/Yeast laboratory trials

Olfactometer Y-tube tests were conducted in the laboratory of all the isolates grown in nutrient agar, nutrient broth plus 10 mm phloem and bark discs, nutrient broth plus phloem filtrate, and sugar ferment solution. None of the isolates were shown to be statistically significant in the attraction of *H. ater* adult beetles although isolate numbers 1, 5, 6 ,7 and 9 did result in high numbers of positive reponses.

Isolate	Description	Identification
1		Yeast
2	Gram –ve small short rods	F. Enterobacteriaceae G. Edwardsiella
3		Yeast
4	Gram –ve small rods	F. Enterobacteriaceae G. Edwardsiella
5	Gram –ve small rods	F. Enterobacteriaceae
6	Gram –ve small rods	F. Enterobacteriaceae
7	Gram –ve small rods	G. Pseudomonas/Alcaligenes
8	Gram –ve small rods	F. Enterobacteriaceae G. Klebsiella
9	Gram –ve small rods	F. Enterobacteriaceae
10	Gram –ve small rods	F. Enterobacteriaceae G. Edwardsiella
11	Gram –ve small rods	F. Enterobacteriaceae G. Citrobacter
12	Gram –ve small rods	F. Enterobacteriaceae G. Edwardsiella

Table 6.1. Bacteria and yeast isolates associated with *H. ater* adult beetles

6.4.1.3. Bacterial/Yeast field trials

There was no significant difference in number of entry holes or catches of *H. ater* in response to the isolates for all field trials including a) isolates in nutrient agar, nutrient broth plus phloem and bark discs, nutrient broth plus phloem filtrate in McCartney bottles attached to billets (Appendix 17 and 18); b) isolates in sugar ferment solution in sticky bottle traps (due to the traps being an inefficient capturing method); and c) inoculated billets (Appendix 19).

6.4.1.4. Sterilisation trials

6.4.1.4.1. Sterilisation trial 1

All of the treatments were shown to be statistically non significant when compared on the basis of entry holes and number of beetles per cm^2 and cm^3 of *P. radiata* material (Appendix 20). The number of entry holes and the number of beetles recorded on and within all billet treatments was quite low, indicating that the trial was established at a time when the beetle population was low or the presence of the plastic bag inhibited beetle access.

6.4.1.4.2. Sterilisation trial 2

The treatment of a control billet buried directly in field soil without the use of a bag was shown to be significantly different from all other treatments when compared on the basis of number of entry holes and adult beetles per cm^2 and cm^3 of *P. radiata* material with p-values of 0.0021, 0.0002, 0.0085, and 0.0013 respectively (Appendix 21). The other treatments were all buried in bags which must have inhibited beetle access.

When the results were reanalysed including only the billets buried in bags a difference was detected. The number of entry holes per cm^2 and cm^3 of *P. radiata* material recorded on frozen billets was significantly different to that recorded on sterilised and control billets. The mean number of entry holes recorded on the frozen billets was 0.0096 per cm^2 of *P. radiata* material compared to 0.0027 and 0.0029 for the sterilised and control billets respectively. No differences were detected between sterile sand and field soil or billet type and soil type.

6.4.1.5. Attraction to damaged *P. radiata*

The total number of entry holes recorded and adult beetles present within the frozen and control billets were the same, but when compared on the basis of surface area and volume taking into account the differences in billet size clear differences were evident. The differences in the number of entry holes per cm^2

and cm^3 of *P. radiata* material and adult beetles per cm^3 of *P. radiata* material recorded from frozen billets was shown to be statistically significant with p-values of 0.0311, 0.0108, and 0.0381 respectively from those recorded on the control billets (Appendix 22). The number of entry holes and adult beetles recorded from frozen billets were three times the numbers found on control billets when billet size was taken into account. A replicate difference was also noted which is most likely due to less beetles present at site two, perhaps due to lower quantities of available breeding material.

Visual differences between the frozen logs and the control logs was noted with the development of microbial growth on each cut end of the frozen billets. A green/black coloured fungal growth (possibly a blue-stain fungal species) following the veins of the wood was observed. The bark of the frozen logs was also very easy to remove, with the freezing treatment having accelerated tissue breakdown, although the bark was still intact. At the completion of the trial two of the frozen billets contained galleries with larvae just hatching and beginning to feed compared to just one of the control billets containing larvae.

6.4.2. Fungal Associations

6.4.2.1. Scanning electron microscopy

After detailed investigation with the scanning electron microscope no specialised mycangial pits were observed for *H. ater*. Surface pits were present on the head, pronotum and elytra, and contained rounded fungal spores. These structures can clearly be seen in Plates 6.1, 6.2 and 6.3.

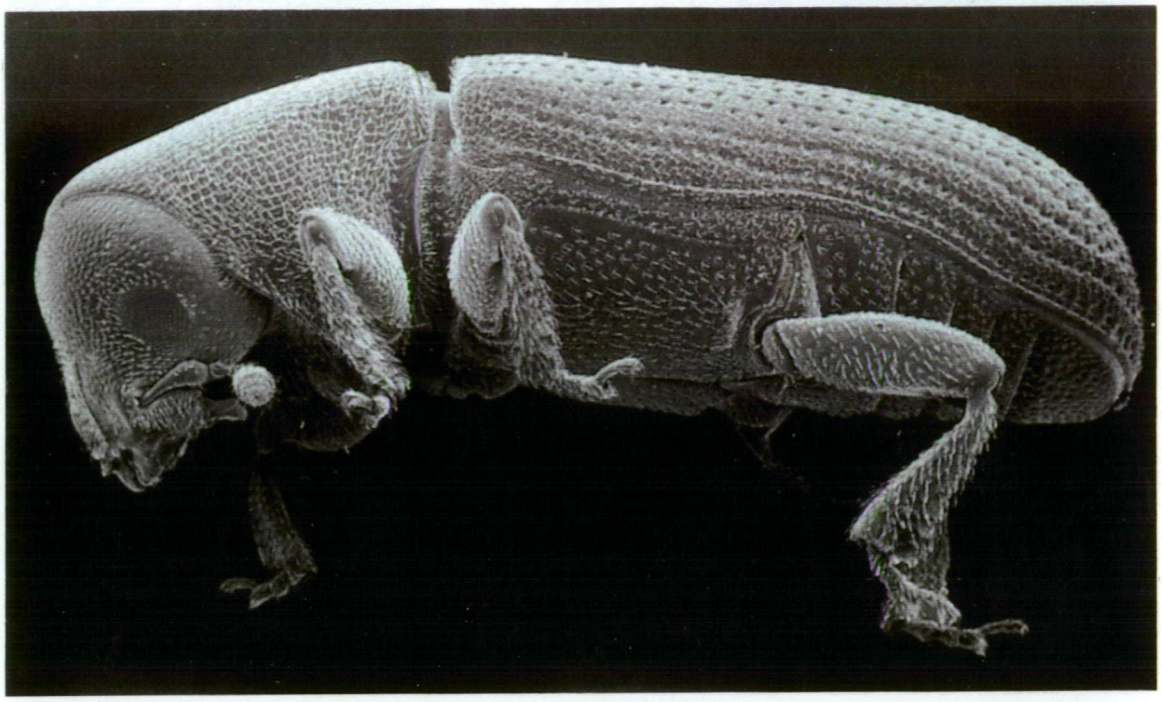

Plate 6.1. Surface pits on the head, pronotum and elytra of adult *H. ater* beetle (x 21)

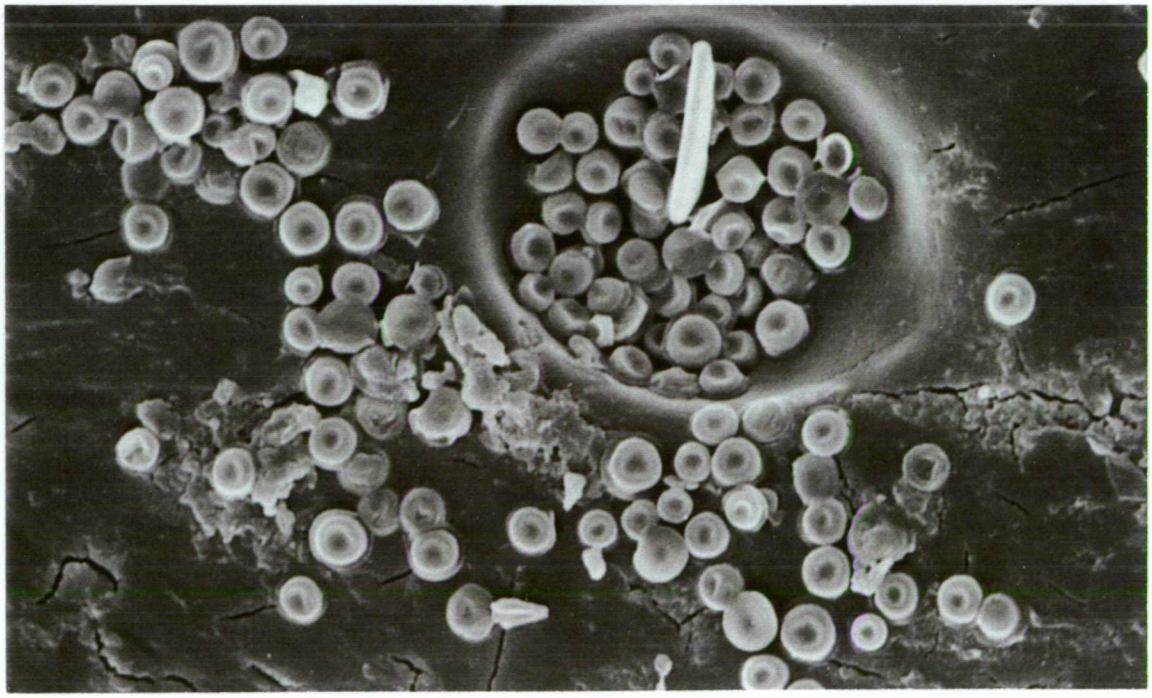

Plate 6.2. Surface pits containing rounded fungal spores on the elytra of *H. ater* adult beetles (x 1550)

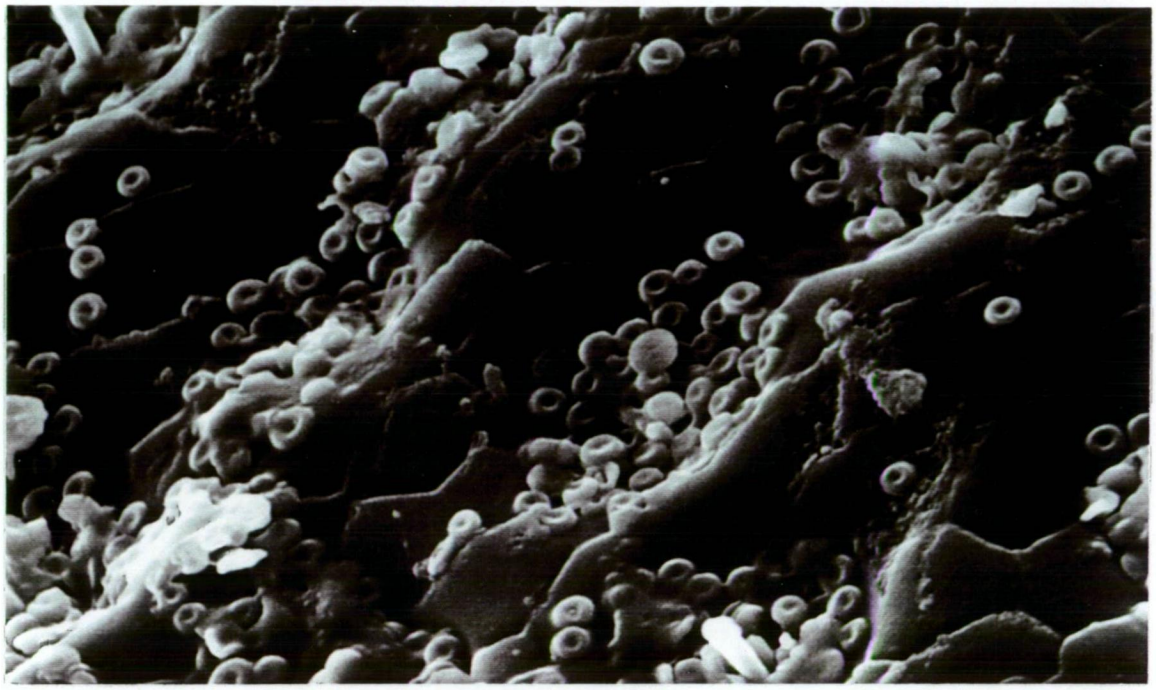

Plate 6.3. Surface pits containing rounded fungal spores on the pronotum of *H. ater* adult beetles (x 1250)

6.4.2.2. Fungal isolations and laboratory olfactometer trials

The most commonly associated fungal organism isolated from *H. ater* was *L. lundbergii*. This fungal organism was also isolated from *H. ater* tunnels so it is possibly involved in the creation of suitable phloem tissue for larval feeding.

Pupal chambers of *H. ater* are characteristically lined with a white mat of fungal hyphae, consisting of *L. lundbergii*. This lining is not found in galleries after adult emergence indicating that it has been devoured by pre-emergent beetles.

Numerous other fungal organisms were isolated from *H. ater* adult beetles but not as consistently as *L. lundbergii*. The other fungi isolated included numerous mucor lower fungi, *Penicillium* spp., *Metarhizium* spp., *Trichoderma* spp., and a coleomycete *Aschersonia* spp. The scope of the project did not allow for further identification of the less commonly associated fungal organisms.

The *H. ater* response to *L. lundbergii* trialed in the laboratory olfactometer Y-tube was shown to be statistically non significant.

6.4.2.3. Influence of *H. ater* on the growth of *Leptographium lundbergii*

From measurements and counts made on the growth of *L. lundbergii* no quantitative difference could be determined between the different agar treatments. From qualitative observations, the autoclaved and nonautoclaved plates had better growth overall than the control plates, while the autoclaved plates had better growth than the nonautoclaved plates. On two of the nonautoclaved plates a halo was evident around the beetle pieces inhibiting *L. lundbergii* growth. From this simple experiment it was concluded that the addition of autoclaved and nonautoclaved *H. ater* to the MEA did not influence the growth of *L. lundbergii.*

6.4.2.4. Entomopathogenic fungal trials

The results of entomopathogenic fungal trials 1 and 2 are shown in Table 6.2 and 6.3 respectively. Both pathogenic fungi worked best at the higher temperature of 20°C, resulting in only a small number of deaths at 10°C. At 20°C *M. anisopliae* applied in the heavier dose level of treatment B was effective in resulting in the death of 80% of beetles within ten days. The *B. bassiana* culture appeared to be ineffective against *H. ater*, requiring several weeks to cause death.

The results of entomopathogenic fungal trial 3 are shown in Table 6.4. The peanut oil treatment and the peanut oil control resulted in 100% death, this is presumed to be due to the peanut oil and not the fungus. The *M. anisopliae* spores applied to the bark successfully resulted in the death of 70% of *H. ater* within 17 days and 95% death within five weeks.

Treatment	*B. bassiana*			*M. anisopliae*		
	10 days	24 days	38 days	10 days	24 days	38 days
Treatment A at 10°C	0	1	1	0	0	0
Treatment B at 10°C	0	1	1	0	0	0
Treatment A at 20°C	0	0	0	7	8	8
Treatment B at 20°C	1	2	6	9	9	9

Table 6.2. Number of *H. ater* deaths recorded over time for each treatment (Entomopathogenic fungal trial 1)

Treatment	*B. bassiana*			*M. anisopliae*		
	10 days	24 days	38 days	10 days	24 days	38 days
Treatment A at 10°C	0	1	1	0	0	0
Treatment B at 10°C	0	0	1	0	0	0
Treatment A at 20°C	2	2	2	8	8	8
Treatment B at 20°C	0	0	2	8	8	8

Table 6.3. Number of *H. ater* deaths recorded over time for each treatment (Entomopathogenic fungal trial 2)

Treatment	10 days	17 days	38 days
M. anisopliae on bark at 20°C	0	6	9
M. anisopliae on bark at 20°C	0	8	10
M. anisopliae in peanut oil on bark at 20°C	10	10	10
M. anisopliae in peanut oil on bark at 20°C	10	10	10
Control at 10°C	0	0	0
Control at 20°C	0	0	1
Control peanut oil on bark at 20°C	2	4	10

Table 6.4. Number of *H. ater* deaths recorded over time for each treatment (Entomopathogenic fungal trial 3)

6.5. DISCUSSION

6.5.1. Bacterial and Yeast Associations

Ten bacteria belonging to the family Enterobacteriaceae and two yeasts were found in constant association with *H. ater* beetles. The bacterial family Enterobacteriaceae are distributed world wide being found in soil, water, fruits, vegetables, animals, man and insects (Krieg 1984). These isolates were trialed in the laboratory (using a Y-tube olfactometer) and field trials but all treatments were shown to be not significantly different. The bacteria and yeasts isolated from *H. ater* do not appear to be involved in the attraction process.

Exclusion of the normal microflora of both the *P. radiata* material by sterilising billets and the soil by using sterile sand did not influence *H. ater* attractiveness. No differences were detected between sterilised, surface sterilised or control billets buried in both field soil and sterile sand within bags for both trials. The only treatment which exhibited a higher number of *H. ater* entry holes were billets subjected to freezing. The use of heavy duty plastic bags inhibited

beetle access to billets with control billets buried without bags exhibiting significantly higher attraction than billets buried in bags.

The higher number of *H. ater* beetles attracted and supported by frozen billets must be due to the freezing treatment having damaged the cells within the *P. radiata* phloem resulting in accelerated tissue breakdown making the material more suitable for *H. ater* indicated by the advanced brood development detected in the damaged billets. Such conditions of accelerated breakdown indicative of the ease of bark removal would appear to be very suitable for *H. ater* development and would also happen naturally within the control billets but after a longer time frame in the field under Tasmanian conditions. The attractiveness of the damaged *P. radiata* material was shown in Chapter Five not to be due to an increase or change in the volatile compounds released and detected by adult beetles. This suggests the involvement of microorganisms.

Future work is recommended to investigate the different microorganisms associated with *P. radiata* material, as bacterial and yeast isolates associated with *H. ater* were shown not to be involved in host selection and attraction. Microorganisms associated with *H. ater* adult beetles was the primary focus of this investigation.

6.5.2. Fungal Associations

Of the numerous fungi isolated from *H. ater* the most commonly associated fungal organism was *L. lundbergii*, which possessed characteristically branched upright conidiophores with penicillate branches in their upper portion (Barnett 1962). *L.* serpens, *L. truncatum* and an unidentified *Leptographium* species have been isolated from *H. angustatus* and *H. ligniperda* (Wingfield & Swart 1989). The Australian ambrosia beetle, *P. subgranosus* also vectors *L. lundbergii* (Webb 1945). Laboratory trials with the fungal isolates grown on agar media showed them not to be attractive to *H. ater*. The *Leptographium* species associated with *H. ater* beetles was also isolated from their tunnels. The *Leptographium* species may play a role in host selection as fungi associated with

other bark beetles result in increasing the level of host monoterpenes (Shrimpton 1973, Raffa & Berryman 1982, Lieutier et al 1989). The attractiveness of the *Leptographium* species should have been trialed against *H. ater* when grown on *P. radiata* material. The presence of the *Leptographium* species in *H. ater* tunnels and lining pupal chambers suggests that the fungal organisms play a significant role in conditioning the host for brood development and in the provision of food. This area is recommended for future study.

SEM showed that the fungal spores were carried primarily in surface or mycangial pits on the head, pronotum and elytra.

The simple experiment involving the addition of autoclaved and nonautoclaved adult *H. ater* beetles to the MEA used to culture *L. lundbergii*, did not influence the growth of the fungus.

The introductory work with the two pathogenic fungi *B. bassiana* and *M. anisopliae*, although based on very simple experiments requiring further investigation, revealed several important results. Both fungal agents performed best at the higher temperature of 20°C, being fairly ineffective at 10°C.

M. anisopliae appears to be effective against *H. ater* adult beetles when applied directly to the beetles or indirectly on the bark, at a temperature of 20°C. Thus for practical use in the field *M. anisopliae* could potentially be effective over the summer period although temperatures in the study area rarely get to over 20°C. It thus seems highly unlikely that this fungus could be used as a control means in cooler parts of Australia. *B. bassiana* is not as effective against *H. ater*. The pathogenic fungi *M. anisopliae* and *B. bassiana* trialed with *H. ater* have the potential to be effective against the adult beetles at high temperatures. *B. bassiana* was more effective than *M. anisopliae*, but the limiting factor for potential field application would be temperature. The use of peanut oil as a means of dispersing the fungal spores as a spray would require further analysis.

CHAPTER 7

HOST SELECTION AND ATTRACTION - SUMMARY

The subterranean habitat occupied by *H. ater* contrasts markedly with the many species of bark beetles which attack host trees above the ground. The importance of the numerous interacting factors within the soil environment and their influence on the host selection and attraction of *H. ater* cannot be underestimated.

Adult *H. ater* beetles invade fresh *P. radiata* material within the first two days of harvest with the number of beetles arriving reaching a peak within seven to 14 days. Fresh host material is invaded following harvesting at any time during the year. Both male and female *H. ater* beetles arrive at new host material simultaneously in equal numbers. Female *H. ater* beetles initiate entry through the bark playing an important role in host colonisation by constructing the egg gallery with the male following behind. Mating may take place on the bark, during entry or within the gallery.

The only slash items attacked by *H. ater* are those that are in direct contact with the soil either lying on the soil surface or partially buried within the soil. Presence of the bark-soil interface is a major key in the susceptibility of slash items to attack by *H. ater*. Following harvesting procedures the majority of the stumps and logging slash in direct contact with the soil are attacked by *H. ater* and utilised as breeding sites.

H. ater does not invade host material completely buried below the soil surface, only invading host material buried level with the soil surface or with some of the material protruding above the soil surface. *H. ater* will invade *P. radiata* host material incorporated into the soil to depths of 80 cm below the soil surface as long as some of the material protrudes above the soil surface.

H. ater is capable of invading *P. radiata* material of any bark thickness being able to complete their entire life cycle within a range of bark thicknesses

from two to 24 mm. There is a direct relationship between bark thickness and the number of beetles invading the bark.

The moisture content of *P. radiata* material has a significant effect on its attractiveness to *H. ater* adult beetles and suitability for brood development. *P. radiata* material with bark and wood moisture contents of less than 50% are unattractive to *H. ater* and unsuitable for brood development. Stumps support *H. ater* development for up to two years after harvesting operations. Logging slash can remain attractive up to three months following harvesting but has the potential to become attractive and suitable for brood development for a much longer period following a rise in bark moisture content. Dry *P. radiata* which is unattractive to *H. ater* was invaded following the exposure of dry billets to a water soaking treatment which caused a rise in bark moisture content. The soaking treatment did not influence the residual volatile monoterpene compounds present in the dry material but may aid their release resulting in *H. ater* attraction and ultimate invasion due to a suitable moisture content.

A longer duration of attractiveness was shown by sustained high moisture content billets with restricted drainage which demonstrates the longer duration of suitability of stumps and slash within the soil which can maintain a suitable moisture content compared to harvested slash with its smaller diameters and bark thicknesses will dry out quicker on the soil surface.

It was shown that host *P. radiata* chemical compounds play an important role in *H. ater* host selection and attraction. The attractive ability of *P. radiata* bark to *H. ater* was demonstrated by the attraction of beetles to the non host *Acacia* and *Eucalyptus* spp., when wrapped in *P. radiata* bark. Also in laboratory olfactometer trials *P. radiata* bark resulted in 100% attraction of *H. ater* adult beetles. One of the major monoterpenes in *P. radiata* material, α-pinene resulted in the attraction of *H. ater* to *Acacia* spp. billets coated in α-pinene.

The main monoterpenes present in fresh, frozen and dry *P. radiata* material detected by GC-MS analysis were α-pinene, β-pinene, β-phellandrene, δ-3-carene, limonene, myrcene and sabinene. Over time the general levels of the

main monoterpenes decline and those of the oxidised monoterpenes including terpinolene, fenchone, camphor, pinocarveol, pinocamphone, isopinocamphone, terpinene-4-ol and α-terpineol increase. It was found that large genetic differences existed within *P. radiata* material in terms of its monoterpene complex.

The same general monoterpene pattern as found in *P. radiata* material was found in the analysis of feeding *H. ater* beetles. However the production of oxidised monoterpenes was greatly enhanced.

H. ater adult beetles must be able to detect the primary host monoterpenes and derivatives released by *P. radiata* material and respond to the subtle changes which occur. The attraction of *H. ater* to damaged *P. radiata* and seedlings may be associated with an increased release of host monoterpenes as found by Hodges and Lorio (1975), although GC-MS analysis conducted during this study did not detect any difference in monoterpene composition. As both beetle sexes arrive simultaneously no one sex is responsible for host selection, with both *H. ater* sexes exhibiting clear primary attraction as a result of the release of host volatiles from the large source of bark in contact with the soil. Invasion is only noted in host material which protrudes above the soil surface which enables the beetles to detect the volatiles being released.

The attack of *H. ater* primarily on logging residues and seedlings does not require a mechanism for overcoming host resistance. After the initial arrival of a few pioneer beetles, primary bark beetles such as *Dendroctonus* species, which attack living mature trees utilise insect produced compounds in addition to host produced compounds to cause mass aggregation on the host to overcome host resistance allowing successful colonisation. The involvement of insect produced compounds in the host selection process of a soil dwelling secondary insect would be of little use in the host attraction process.

The invasion of *H. ater* reaches a peak within the first seven to ten days. The changes in compounds detected from feeding *H. ater* beetles that occur after this time frame are the oxidised monoterpenes fenchone, camphor,

isopinocamphone and an unknown compound increasing over time as feeding progresses. These compounds are released in *H. ater* frass. The oxidised monoterpenes are also produced naturally from *P. radiata* material over time but their presence is greatly enhanced by the action of *H. ater* feeding. The oxidised monoterpenes and the unknown compound detected in *H. ater* frass are still present in quite small quantities being produced after the majority of *H. ater* beetles have arrived at the host material. The habitat of *H. ater* would inhibit the diffusion of these compounds and they would not appear to compete with the mass release of volatiles from the harvesting residue above and within the soil. The small changes detected in the oxidised monoterpenes and the production of the unknown compound by the action of feeding beetles could play a role within each host as messengers involved in mate pairing or inhibiting further colonisation but not in the primary attraction to the host.

The detection of no major unique compounds being produced by *H. ater* in significant quantities aside from host monoterpenes and derivatives demonstrate that primary bark beetle pheromones or insect produced compounds are not directly involved in the attraction process. The field and laboratory assessment of potential volatile compounds which may play a role in *H. ater* host selection and attraction did not increase attractiveness to *P. radiata* billets. No one compound resulted in an enhanced attraction of *H. ater* adult beetles.

The ability of only males to stridulate may be involved in courtship and mate pairing once both beetle sexes have arrived at suitable host material and could well assist in the defence of the female and the resulting brood gallery from other *H. ater* males or beetle pairs.

A number of microorganisms including bacteria, yeast and fungi were isolated from adult *H. ater* beetles. Ten bacteria belonging to the family Enterobacteriaceae and two yeasts were commonly associated with *H. ater*. A number of lower and higher fungal organisms were also isolated from adult *H. ater* beetles, with *L. lundbergii* found in constant association. The fungal isolates

were observed in surface pits on the head, pronotum and elytra of both male and female adult *H. ater* beetles.

From both field and laboratory olfactometer trials the bacterial, yeast and fungal isolates from beetles did not appear to play a role in *H. ater* host selection and attraction. The attraction of *H. ater* to *P. radiata* billets damaged by freezing suggested the involvement of microorganisms but the involvement was not proven due to study time restraints. The initial hypothesis that the host selection and attraction process could involve the beetles plus the host responding to the action of microorganisms namely yeasts, bacteria and fungi does not appear to be true in terms of the microorganisms associated with *H. ater*. However, the role of microorganisms isolated from *P. radiata* host material in host attraction was not extensively investigated in this study.

Being a soil dwelling insect *H. ater* would encounter a wide range of microorganisms, but one would expect that the microorganisms found in constant association with the beetles would play some role, even if it is not host selection and attraction. *L. lundbergii* isolated from adult *H. ater* beetles was also present in their galleries, so may play a role in host colonisation and brood development and nutrition. This aspect was not tested in the current study.

CHAPTER 8 NEMATODE ASSOCIATION

8.1. INTRODUCTION

This study was undertaken to isolate and determine the taxonomic identity, parasitic status and potential use of nematodes associated with *H. ater* as possible control measures.

Nematodes are one of the many biotic factors which adversely affect bark beetle populations (Massey 1974), being just one of many organisms associated with bark beetles around the world (Poinar 1975, Dahlsten 1982). Many bark beetles are infected by parasitic nematodes which can result in reduced egg production, sterility, reduced life span, altered behaviour and death in the host insect (Massey 1960, Nickle 1963, Massey 1964, Poinar 1975, Kaya 1984). Poinar (1975) lists *H. ater* as the host for 16 nematode species.

8.2. LITERATURE REVIEW

8.2.1. General

Nematodes are described by Poinar (1983) as "appendageless, non segmented, wormlike invertebrates possessing a body cavity and a complete digestive tract".

In general, there are three main stages in the development of nematodes: eggs, larvae (including four growth stages), and adults (Steinhaus 1949).

Nematodes are capable of killing, sterilising or debilitating a large range of insects, including bark beetles (Nickle 1974). In general, insects show little external evidence of nematode parasites with their presence resulting in internal effects influencing growth, activity and behaviour (Welch 1965). The effects of nematodes on bark beetles may include reduced fecundity (Nickle 1963, Massey 1974), delayed emergence (Nickle 1963), reduced adult longevity (Massey 1964) and reduced flight activity (Ashraf & Berryman 1970b).

8.2.2. Insect-Nematode Relationships

Different relationships may exist between insects and nematodes, which include phoresy, facultative and obligate parasitism.

8.2.2.1. Phoretic relationships

Phoretic relationships are often regarded as the most common type of relationship between insects and nematodes, where the insect serves only as a means of transport for the nematode (Poinar 1975). This type of relationship often occurs when the same ecological niche is shared. Nematodes can be carried externally on any part of the insects body, as well as internally in the reproductive tract of both sexes, the trachea, gut or Malpighian tubules (Poinar 1975). Nematode associates are found among many different nematode families including Aphelenchidae, Aphelenchoididae, Cephalobidae, Chambersiellidae, Cylindrocorporidae, Diplogasteridae, Dorylaimidae, Mononchidae, Neotylenchidae, Panagrolaimidae, Plectidae, Rhabditidae, and Tylenchidae (Nickle 1974). The insect host is rarely affected by the nematodes presence, and it assists in the dissemination of the nematodes. It is unknown what role the nematodes might play in creating a more suitable habitat for the insect.

8.2.2.2. Facultative parasitism

Facultative parasitism occurs when nematodes parasitise healthy insects yet still retain the ability to complete their life cycle in the hosts environment (Poinar 1975). Facultative parasitic nematodes can be found in the body cavity, intestine, Malpighian tubules, pharyngeal glands, trachea, and colleterial glands. Most obligate parasitic nematodes receive nourishment at the expense of the host and their growth in the insect may involve an increase in width, a moult, or continuous development. Nourishment during the non-insect cycle is obtained from fungi, bacteria, and higher plants. The damage these nematodes inflict on their hosts varies tremendously, and can result in the death of the host (Poinar 1975).

8.2.2.3. Obligate parasitism

The obligate nematode parasites of insects have either no free-living stages that receive nourishment or complete a separate life cycle in the hosts environment. At most, there is a maturation period in the host environment which consists of one or more moults, mating and oviposition, but a living insect is needed to complete the cycle. These nematodes generally occur in the body cavity of the host, but may occur in the intestine or reproductive system. The effect the obligate nematode parasites have on their insect hosts ranges from negligible to sterilisation and death. Most nematode parasites of bark beetles are obligate parasites that do not kill their hosts (Kinn 1984).

8.2.3. History

The first invertebrate-parasitic nematode was recorded by the French naturalist Reaumur in 1742 during an investigation of bumblebees, and now known as *Sphaerularia bombi* Duf. (Reaumur 1742). In 1890, von Linstow discovered the nematode *Allantonema diplogaster* (*Contortylenchus diplogaster* Linstow) associated with *Tomicus typographus* (*I. typographus*) (Massey 1974).

Fuchs (1915) initiated a little known field of nematology dealing with nematode parasites and associates of bark beetles (Massey 1974). Numerous species of Tylenchoidea, Aphelenchoidea, and Rhabditoidea were described, including several new genera. Yatsentkovskii (1924) in the USSR provided evidence that a small number of nematodes could cause castration of bark beetle hosts, and heavy infections resulted in death (Massey 1974). Infection with a *Parasitylenchus* species caused the sterilisation of *S. multistriatus* and *Scolytus scolytus* Fabricius (Oldham 1930 cited in Massey 1974).

Early in this century, workers in Germany and other countries began to notice that bark beetles were being parasitised by nematodes (Nickle & Welch 1984). Dissections of parasitised beetles are very spectacular, often revealing numerous larval nematodes and one or more females free in the hemolymph of the body cavity of each adult bark beetle (Nickle & Welch 1984). Ruhm (1956) did

extensive early work on the taxonomy of nematode parasites and associates of bark beetles. In 1963, Nickle completed a PhD study on bark beetle nematodes and worked on the infective stage female of *Contortylenchus* (Nickle & Welch 1984).

8.2.4. Parasitic Nematodes

The life cycles of parasitic nematodes of bark beetles are closely synchronised with those of their hosts (Kaya 1984), and an individual bark beetle may be infected by more than one nematode genus or species (Massey 1974). Nematodes invading the body cavity and tissues of insects are included in the families Tetradonematidae, Mermithidae, and Allantonematidae (Steinhaus 1949). Nematodes attacking bark beetles belong to the families Sphaerulariidae, Aphelenchoididae, and Rhabditidae (Kaya 1984, Poinar & Caylor 1974). Nematode genera with bark beetles as their usual host insect include *Sphaerulariopsis* Wachek, 1955; *Allantonema* Leuckart, 1884; *Bovienema* Nickle, 1963; *Contortylenchus* Ruhm, 1956; *Neoparasitylenchus* Nickle, 1967; *Parasitylenchus* Micoletzky, 1922; and *Sulphuretylenchus* Ruhm, 1956 (Nickle 1974).

Insect parasitic nematodes are primary, obligate parasites with most being true parasites which do not kill their host (Massey 1974, Nickle 1974). They actively search out larval, pupal, or adult stages of insects and enter via the oesophagus, anus, as well as the cuticle, and once inside the host, the nematode obtains nourishment from the hemolymph (Nickle 1974). Thus reduced egg production or sterility is common in parasitised insects (Nickle 1974). Infective stages of entomophagous nematodes are usually released into the habitat of the larval host insect, for example in bark beetle frass.

8.2.5. Allantonematidae

Allantonematidae comprise a large group of insect parasitic nematodes, which have a life cycle distinctly different from that found in any other group

(Steinhaus 1949). The effect of infection by allantonematid nematodes on their bark beetle hosts may be negligible or result in sterility and even death (Poinar & Caylor 1974).

The adult gravid females occupy the body cavity of the insect, frequently in small numbers or, often, one per host. Eggs are either deposited in the body cavity of the host or they hatch before deposition. In either case, the young larvae commence their development in the host insect, moulting once or twice, depending on the species, and then escape from the host. This is accomplished either by entering the alimentary tract and passing out through the anus or by entering the female reproductive system and passing out through the genital aperture. In most cases, both male and female insects are infected by the nematodes. The free-living stage, usually of short duration, is passed wherever the host insect undergoes its early development. During this period, the nematodes moult at least once, in most cases probably twice, and become adults.

Upon entering a new host, the female nematode increases greatly in size. When fully grown, the female is usually curved ventrally and assumes a sausage like shape; there are, however, exceptions to this. If the female does not actually lay her eggs in the body cavity of the host, her uterus becomes distended with developing eggs and larvae, which fill a large part of her body, and some of her organs degenerate. Usually the larvae pass out through the vulva of the female into the body cavity of the insect. In some nematode species the size of the female does not increase enough to provide space for the expanding reproductive organs. As Christie (1941) points out in his discussion of the group, there are several deviations from this typical life cycle. In some cases, the males as well as the females enter the body cavity of the insect. Sometimes neither the adult males nor the females become parasitic, this role being taken over by the larval stages. A few species have heterogeneous life cycles, having parthenogenic and gamogenetic generations (Steinhaus 1949).

8.2.6. Parasitic Nematode Infection of Bark Beetles

The bark beetle *I. typographus* is parasitised by at least two species of allantonematids, *Aphelenchus diplogaster* Linst., and *Parasitylenchus dispar* subsp. *typographi* Fuchs (Steinhaus 1949). *P. dispar* infection of *I. typographus* results in reduced fat body and gonads, reduced egg production and a slower egg maturation rate, and death (Fuchs 1915, Poinar & Caylor 1974).

The bark beetle *Pityogenes bidentatus* Hbst. has been found to harbour *Aphelenchulus tomici* Bov. which, like *A. diplogaster*, passes out of its host through the anus and undergoes its free-living development in the frass of the beetles galleries (Steinhaus 1949).

Infection of *Ips perturbatus* Eichoff with *Parasitorhabditis obtusa* Fuchs resulted in damage to the midgut epithelium (Tomalak et al 1989).

I. confuscus is sterilised by *Sulphuretylenchus* sp. and the nematode parasite *Contortylenchus elongatus* Massey reduces the number of eggs laid by the beetle (Nickle 1963). Massey (1962) noted an altered gallery construction behaviour and reduced fecundity in *I confuscus* parasitised by *C. elongatus* (Poinar & Caylor 1974). *Ips lecontei* Sw. is also parasitised by *C. elongatus* (Massey 1974).

The nematodes *Parasitorhabditis hastulus* Massey, *Contortylenchus grandicolli* Massey, and *Mikoletzkya calligraphi* Massey were found in association with adult *I. grandicollis* beetles (Stone 1990).

Massey (1956) found that *D. rufipennis* showed reduced egg production when infected by *Sphaerulariopsis dendroctoni* Massey and *Contortylenchus reversus* Thorne (Poinar & Caylor 1974). These two nematodes are also associated with *D. pseudotsugae* (Furniss 1967).

Dendroctonus adjunctus Bland. is parasitised by *Parasitaphelenchus dendroctoni* Massey, and *D. brevicomis* is parasitised by *Contortylenchus brevicomi* Massey (Massey 1974).

Massey (1964) found *S. ventralis* was sterilised by *Sulphuretylenchus elongatus* Massey, with infection resulting in delayed emergence, limited flight,

construction of short galleries, disruption of the digestive processes, and eventual death of the host (Ashraf & Berryman 1970a, 1970b, Poinar & Caylor 1974).

8.2.7. Nematodes Associated with *H. ater*

Insect-nematode associations have been recorded between *H. ater* and nematode species from the nematode groups, Allantonematidae, Panagrolaimidae, Aphelenchoididae, Diplogasteridae, Mononchidae, Tylenchidae, and Rhabditidae (Poinar 1975).

Poinar (1975) lists *H. ater* as the host for the nematodes,

Allantonema morosum (Fuchs) 1929

Anguilluloides zondagi (Dale) 1967

Bursaphelenchus chitwoodi (Ruhm) 1956

Bursaphelenchus eggersi (Ruhm) 1956

Contortylenchus cunicularii (Fuchs) 1929

Cryptaphelenchus koerneri (Ruhm) 1956

Ektaphelenchus hylastophilus (Fuchs) 1930

Fuchsia thalenhorsti (Ruhm) 1956

Mononchus papillatus (Bastian) 1865

Neoditylenchus panurgus (Ruhm) 1956

Neoparasitylenchus hylastis (Wulker) 1923

Parasitaphelenchus uncinatus (Fuchs) 1929

Parasitorhabditis ateri (Fuchs) 1937

Sulphuretylenchus kleinei (Ruhm) 1956

Parasitylenchus kleini (Ruhm) 1956

Parasitaphelenchus unicinatus (Fuchs) 1929

Parasitylenchus hylastis (Wulker) 1923

Ruhm (1956) found *A. morosum* in *H. ater*, and unlike other Allantonematid species that mature in adult insects, *A. morosum* matures in the beetle larvae. This nematode is ovo-viviparous, and eggs and juveniles can be found in the haemocoel of beetle larvae. It is not known whether the beetle larvae

are killed by the nematode or whether infected beetle larvae develop into adults. One of the reasons for the lack of information is the low incidence of infection in *H. ater* populations. On the average, only 3% of the beetle population are infected, and in many cases most beetle populations are free of infection (Ruhm 1956). The life cycle of *A. morosum* is similar to *Parasitylenchus* and *Contortylenchus*, with the exception that larvae and eggs are produced in the immature stages of the host and are subsequently deposited in the galleries (Ruhm 1956, Massey 1974).

Ruhm (1956) found *P. hylastis* infecting 30 to 45% of *H. ater* adult beetles with an average of ten nematodes per beetle.

Clark (1932) in New Zealand described the presence of nematodes in association with *H. ater* beetles under the elytra, amongst the frass from tunnels, and within the hindgut. In New Zealand the nematodes *P. hylastis*, *Micoletzkya thalenhorsti* Ruhm, *Bursaphelenchus eggersi* Ruhm, and *Parasitorhabditis ateri* Fuchs were found in close association with *H. ater* (Dale 1967). All of these nematodes except *B. eggersi* have been recorded by Ruhm as associates of *H. ater* in Europe (Dale 1967). In New Zealand Dale (1967) suggests that *B. eggersi* replaces *Bursaphelenchus chitwoodi* Ruhm as a phoretic under the elytra of *H. ater*. The nematode *Anguilluloides zondagi* n sp., only recorded from New Zealand, was found in *H. ater* frass.

Mills (1983) describes the attack of *H. ater* by several nematode species, but little is known on their impact and specificity.

8.2.8. Nematodes Associated with *H. ligniperda*

Insect-nematode associations have been recorded between *H. ligniperda* and nematode species from the nematode groups Diplogasteridae, Allantonematidae, Aphelenchoididae, Rhabditidae, and Panagrolaimidae and Poinar 1975 lists *H. ligniperda* as the host for the following nematodes:-

Fuchsia hylurginophila (Ruhm) 1956

Neoparasitylenchus ligniperdae (Fuchs) 1929

Schistonchus ligniperdae (Fuchs) 1930

Parasitorhabditis ligniperdae (Fuchs) 1937

Plectonchus ligniperdae (Fuchs) 1930

8.2.9. Genus *Neoparasitylenchus*

8.2.9.1. Taxonomy

The genus *Neoparasitylenchus* as described by Nickle (1967) has three distinct forms; one free-living sexual generation and one swollen female developing from the mated free-living female.

Free-living female: Stylet well-developed, with basal swellings. Ovary with more cells than *Contortylenchus* and *Allantonema* spp.

Free-living male: Stylet present, not prominent. Spicules and gubernaculum small, tylenchoid.

Parasitic female: Swollen, slightly mobile, usually sausage- or worm-shaped. Stylet not retracted into inner body. Tail terminal with or without papilla. Oviparous or ovoviviparous. Ovary and oviduct intertwined irregularly, crowded together anteriorly; uterus long, wide, filled with eggs or larvae.

The genus *Neoparasitylenchus* Nickle, 1967 is described by Siddiqi (1986) in the following format.

Entomoparasitic forms: No generative cycle in host.

Entomoparasitic female: Body obese, sausage- or worm-shaped, usually curved, inert or slightly mobile, usually 0.5-2.5 mm long. Cephalic region overgrown by body enlargement. Body white, turning yellowish brown to light brown due to metabolic products. Stylet generally 13-16 μm long, not sunken into body. Vulva subterminal. Uterus enormous, with numerous eggs and juveniles; ovary coiled, pushed into anterior region. Anus terminal or subterminal, tail end usually obtusely rounded, with or without a peg-like projection. Oviparous or ovoviviparous. Juveniles with weakly developed gonad leave host shortly before third and last moult, and develop to adults in a few days.

Partially free-living forms: Free life short. Body vermiform, about 0.4-0.7 mm long. Cephalic region continuous or slightly offset. Stylet generally 14-15 μm long, with slight basal thickenings. Orifice of dorsal gland usually at a distance from stylet base. Excretory pore behind nerve ring. Vulva at about 90-95% of body length. Ovary immature but with more cells than in *Allantonema* and *Contortylenchus*. Postvulval uterine sac rudimentary or absent. Tail conoid, pointed or minutely rounded. Fertilised female invades hosts larva. Male about the size of the female. Spicules small, cephalated. Gubernaculum present. Bursa distinct, enveloping entire tail.

Massey (1974) considered *Neoparasitylenchus* a junior synonym for *Parasitylenchus*, a view not shared by authors such as Nickle (1967) and Poinar (1975) (Siddiqi 1986).

8.2.9.2. Bark beetle hosts

Hosts of *Neoparasitylenchus* are the bark beetles: *Conophthorus*, *Cryphalus*, *Crypturgus*, *Dendroctonus*, *Dryocoetes*, *Hylurgops*, *Hylurgus*, *Hylastes*, *Ips*, *Pitogenes*, *Pityophthorus*, and *Scolytus* (Siddiqi 1986). Wulker (1923) described *Neoparasitylenchus* from several species of *Hylastes*.

8.2.9.3. Effects of *Neoparasitylenchus* infection

Bark beetles infected with *Neoparasitylenchus* may show aberrant gallery construction, reduced longevity, reduced flight activity, reduced fat body, and sterilisation (Kaya 1984).

In the USA, *Neoparasitylenchus avulsi* Massey and *Neoparasitylenchus ovarius* Massey parasitise *Ips* spp. in Alabama and Colorado, respectively (Siddiqi 1986).

Neoparasitylenchus scolyti Oldham was found to parasitise 60% of the *S. scolytus* population in Britain, 40% of which were sterile (Siddiqi 1986).

The nematode parasite *Neoparasitylenchus rugulosi* Schvester induces an altered behaviour in the shot hole borer, *Scolytus rugulosus* Muller (Nickle 1971).

Nickle (1971) observed that parasitised adult beetles construct horizontal galleries, and then die without laying any eggs; compared to healthy beetles which construct galleries parallel with the tree stem, and deposit eggs on both sides of the galleries. The same behavioural response was first noted by Schvester in France in 1957.

The single leaf pinyon cone beetle, *Conophthorus monophyllae* Hopkins is parasitised by *Neoparasitylenchus amvlocercus* sp. resulting in the reduction in fat body, ovaries, and length of life (Poinar & Caylor 1974).

Ruhm (1956) found 6% of the *H. ater* population infected by *N. hylastis* were sterile (Poinar & Caylor 1974).

As mentioned by Kaya (1984) some reports show that infection by Neoparasitylenchs produce no adverse effects on bark beetles. Hoffard and Coster (1976) reported no difference between *Neoparasitylenchus* infected *Ips avulsus* Eichoff and uninfected beetles (Poinar & Caylor 1974). Dale (1967) concluded that *H. ater* was not affected by a neoparasitylenchid infection.

8.2.10. Phoretic Nematodes

Many nematodes are phoretically associated with bark beetles (Massey 1974, Kaya 1984). Phoretic nematodes are transported externally on parts of the beetles body, including under the elytra, between intersegmental folds and on tarsal and tibial joints of the legs; and internally in the gut, Malpighian tubules, reproductive tract and tracheae (Kaya 1984).

Nematode associates of bark beetles have adapted to their host so that only a certain stage such as the resistant form known as dauer larvae are transported to a new environment (Kaya 1984).

8.2.11. Control Agents

Several nematode species have been tested for their efficacy as agents for insect control, but the difficulties of producing large numbers, formulation and successful application has restricted the species of nematode tested in the field

(Webster 1972). Three main factors which work against the successful control of harmful insect populations by nematodes are detailed by Webster (1972) as

(i) resistance of insects to nematode parasitism

(ii) intolerance of the free-living stage(s) of the nematode to low humidities and extremes of temperature and,

(iii) inability to produce large numbers of infective nematodes for use in a control program.

Some nematodes show considerable potential as biological control agents of agricultural and forestry insect pests (Webster 1972). The nematode *Neoplectana carpocapsae* Weiser known as DD-136 has been trialed around the world in the control of insect pests (Welch 1965). DD-136 was sprayed onto pine bark together with a wetting agent, and it was found that it entered the galleries of the pine bark beetle *D. frontalis*, and killed the brood and the adults (Moore 1970). Finney and Walker (1979) detail the unsuccessful control of *S. scolytus* by the application of *N. carpocapsae* as a spray of 5000 nematodes per m^2.

Under controlled laboratory conditions the use of nematode parasites has shown promise upon bark beetle populations (Massey 1974, Kaya 1984). The effect of parasitic nematodes on individual bark beetles of sterilisation, reduced life span, and altered behaviour may play an important role in the natural control of bark beetle populations and may be realised as a biological control agent under field conditions (Kaya 1984). As detailed by Dahlsten (1982) "as with all natural enemies of bark beetles, much biological and ecological background work must be done before their full potential as biological agents can be realised".

8.3. MATERIALS AND METHODS

8.3.1. Isolation of Nematodes from *H. ater* Life Stages and Frass

Life stages of *H. ater* were sourced from *P. radiata* billets established in the field at various locations throughout the plantation, being collected at monthly intervals during the year.

Adult *H. ater* beetles were embedded in paraffin wax and covered in Ringers solution to enable successful dissection of the nematodes from the insect. The nematodes were transferred into clean Ringers solution immediately, washed several times and then placed in a watch glass with Ringers solution, heated gently over a fuel burner until they stopped wriggling. Once dead, the nematodes were washed several times, and then put into the fixative, triethanolamine (TAF), (7 ml formalin, 2 ml triethanolamine, 91 ml distilled water) (Courtney et al 1955).

The same method was employed for isolating nematodes from *H. ater* larval and pupal stages. The free living nematodes were isolated from *H. ater* frass directly into water.

8.4. RESULTS

8.4.1. Nematodes Associated with *H. ater*

Nematodes were discovered within the body cavity of adult, larval and pupal *H. ater* stages, under the elytra of adult *H. ater* beetles and within *H. ater* frass.

8.4.2. *Neoparasitylenchus* Infection of *H. ater*

The fairly large gravid female stage, approximately 1 mm in length, was found throughout the year present within the body cavity of the adult beetle either on or around the digestive system as shown in Plate 8.1. As shown in Table 8.1 of the 488 beetles examined during the period June 1994 to June 1995, 333 were found to be infected and gave a total yield of 1359 adult nematodes. The gravid female stage was present in approximately 70% of adult *H. ater* beetles, with each adult beetle containing 1-14 nematodes, with an average of 2.78 nematodes per beetle for the population as a whole, or 4.08 nematodes per beetle if only the infected beetles are taken into account.

First stage larvae were found within the gravid female nematodes in large numbers within the adult beetles. The first stage larvae or juveniles, were often

observed free in the insect haemocoel having burst from the adult gravid female nematodes. Earlier stage gravid female nematodes with eggs instead of larvae were found within larval and pupal *H. ater* stages.

The nematodes were presumptively identified and subsequently confirmed as belonging to the insect parasitic family Allantonematidae, and the genus *Neoparasitylenchus*, based on the female gravid stage isolated from within *H. ater* larval, pupal and adult stages. Free living male nematodes were required to achieve species identification and although numerous attempts were made, none were found.

In the nematode specimens isolated from *H. ater* the vulva is sub-terminal on the inner side of the ventral curve. The vulva and anus eventually become obscure as the larvae develop and then escape is suspected to be through rupture of the female body. In *Contortylenchus* the body is curved with the ventral surface outwards and the vulva in a deep cleft. The structure of the stylet and pharynx (oesophagus) also agree with *Neoparasitylenchus*. The presence of only a single parasitic generation rules out *Parasitylenchus*.

Date	Jun94-Jun95
Number beetles	488
Number of beetles with gravid females	333
Total number gravid females	1359
Infection gravid females	0.68
Average number gravid females/beetle	4.08
Number of beetles with 1st stage larvae	166
Infection 1st stage larvae	0.34

Table 8.1. Isolation results of the *Neoparasitylenchus* spp. from adult *H. ater* beetles (Appendix 23)

8.4.3. Phoretic Nematodes Associated with *H. ater*

Nematodes were also discovered under the elytra of adult *H. ater* beetles present as a large compacted mass of parallel aligned nematode dauer larvae on the second abdominal tergite as shown in Plate 8.2 and 8.3. The mass was very

hydrophobic. These nematodes being transported by the beetles in the dormant larval stage were unable to be identified.

8.4.4. Nematodes Isolated from *H. ater* Frass

Other nematode species were isolated from *H. ater* frass when searching for *Neoparasitylenchus* free-living males. These included males and females of *Parasitorhabditis* (Rhabditidae) probably *Parasitorhabditis hylurgi* Massey; males and females of *Micoletzkya* (Diplogasteridae) possibly *Micoletzkya pugnea* Massey shown in Plate 8.4, and males and females of *Mesorhabditis* spp.

8.5. DISCUSSION

8.5.1. *Neoparasitylenchus* Infection of *H. ater*

A problem with the taxonomic identification of *Neoparasitylenchus* is that the parasitic female has very few useful taxonomic characters on which to base specific identification. Male nematodes are required to enable further identification to species but despite an intensive search no free-living males were isolated from *H. ater* frass. The *Neoparasitylenchus* spp. isolated is presumed to be the same as *P. hylastis* which is associated with *H. ater* in Germany (Ruhm 1956) and New Zealand (Dale 1967).

A high rate of infection with *Neoparasitylenchus* was experienced by the *H. ater* population throughout the year with the lowest infection levels occurring in the months of July and February as shown in Figure 8.1. The number of nematodes which each adult beetle contained was fairly constant throughout the year as shown in Figure 8.2.

The infection level of adult beetles of 70% is the same as that recorded by Dale (1967) in New Zealand, although the average count of nematodes per beetle is lower in Tasmania. The average of 2.78 nematodes per beetle for the population as a whole, or 4.08 nematodes per beetle if only the infected beetles are taken into account in Tasmania, compares with 3.75 nematodes per beetle for the

population as a whole, or 5.3 nematodes per beetle if only the infected beetles are taken into account in New Zealand (Dale 1967). The results from Tasmania and New Zealand vary markedly from those recorded by Ruhm (1956) in Germany with a beetle infection level of 30 to 45% but an average of 10 nematodes per infected beetle. Dale (1967) explains the difference observed in New Zealand in terms of climatic conditions indirectly influencing life history.

One would expect that the presence of high numbers of nematodes within the haemocoel of individual beetles would have had some effect on their overall existence and performance particularly their reproductive capacity, aside from the nematodes belonging to an insect parasitic family. However the nematodes did not appear to inhibit the black pine bark beetle in any way, and even though this is an entomoparasitic nematode it does not appear to act as a natural control, and would be of no use as a biological control agent. Ruhm (1956) observed reduced gonads in a small percentage of infected *H. ater* beetles.

As noted by Kaya (1984) "The number of nematode species associated with bark beetles is greater than the number of parasitic species". Bark beetles infected with *Neoparasitylenchus* may show aberrant gallery construction, reduced longevity, reduced flight activity, reduced fat body, and sterilisation (Kaya 1984). As mentioned by Kaya (1984) some reports show that infection by *Neoparasitylenchus* produce no adverse effects on bark beetles. Species identification would assist in fully understanding the effect of *Neoparasitylenchus* on *H. ater*. The *Neoparasitylenchus* associated with *H. ater* appears to be a true parasite in that it does not kill its host, and thus its potential use as a control agent is not viable.

8.5.2. Phoretic Nematodes Associated with *H. ater*

Numerous nematode species associated with bark beetles are carried as phoretics under the elytra (Poinar & Caylor 1974, Massey 1974, Kaya 1984), being transported by insects in such a dormant larval stage as those present under the elytra of *H. ater*. Nematode associates of bark beetles have adapted to their

host so that only a certain stage such as the resistant form known as dauer larvae often occuring anhydrobiotically on the beetles are transported to a new environment (Kaya 1984). Dauer larvae are very difficult to identify as the gut is closed down and stains will not penetrate. Time did not allow the culturing of the dauer larvae to adults to enable identification.

8.5.3. Nematodes Isolated from *H. ater* Frass

Nematodes belonging to three other families were isolated from *H. ater* frass. The rhabditid, *P. hylurgi*, which occurs in the hind gut and frass of bark beetles, the diplogasterid, *Micoletzkya* which has been described from *Hylurgops* spp. infesting Red Pine in the USA (Massey 1974), and *Mesorhabditis* spp. Most parasitorhabditids and diplogasterids are phoretically associated with bark beetles, but there are some parasitorhabditids which are parasitic (Kaya 1984).

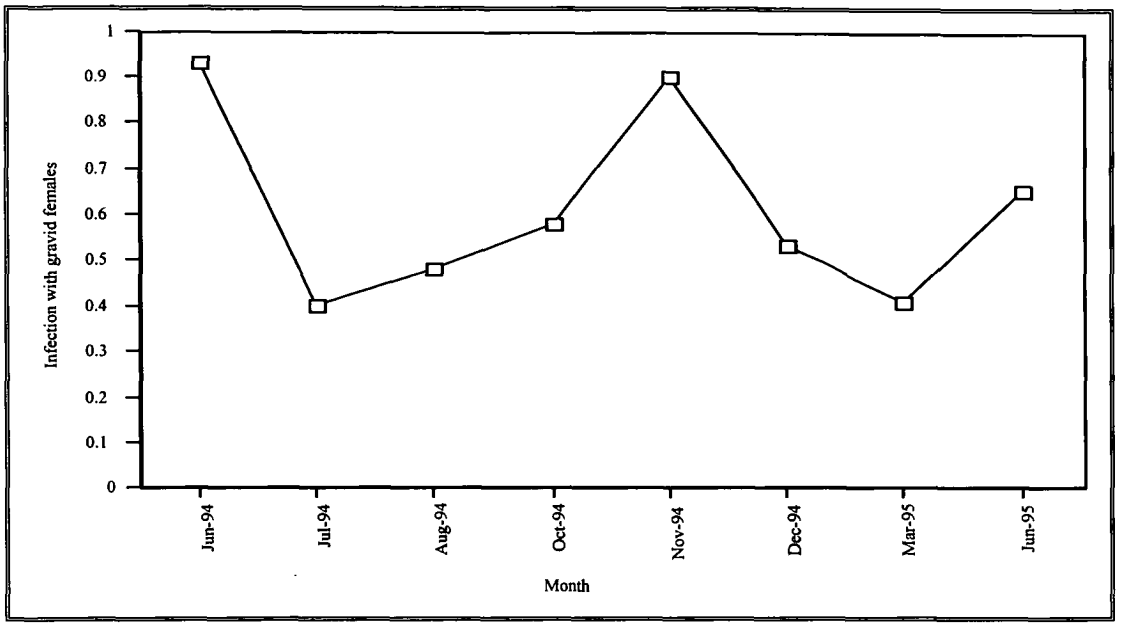

Figure 8.1. Incidence of *H. ater* adult beetles infected with the gravid female stage of *Neoparasitylenchus* spp.

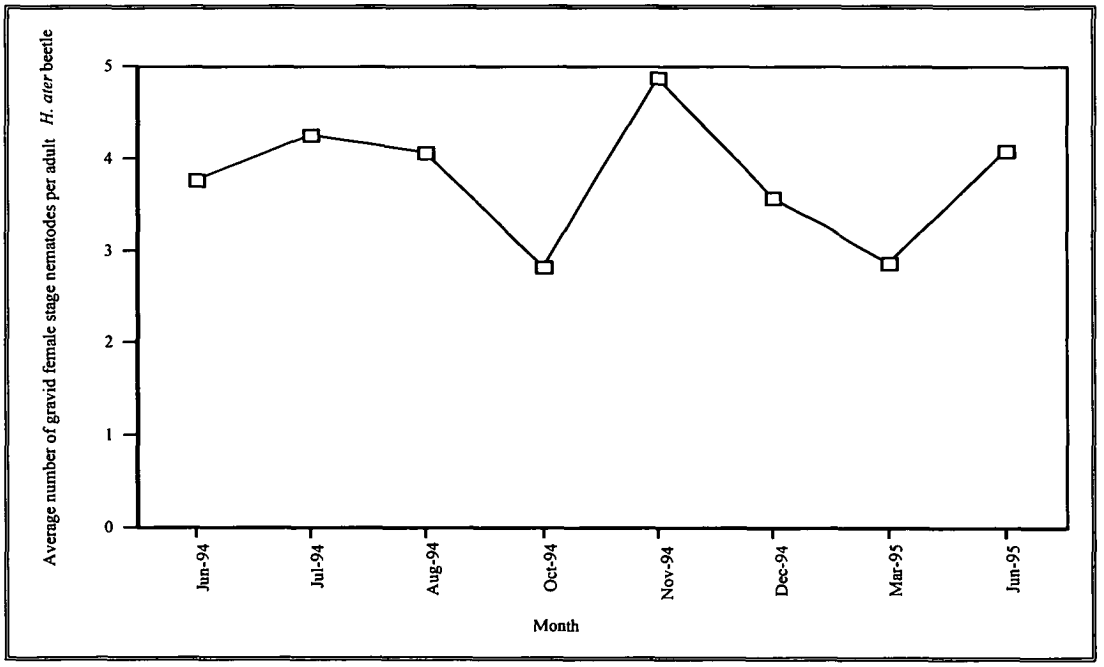

Figure 8.2. Average number of gravid female *Neoparasitylenchus* spp. per adult *H. ater* beetle sampled (n=333)

Plate 8.1. Female gravid stage of the *Neoparasitylenchus* spp. present in the gut of adult *H. ater* beetles. Left x 25. Right x 50.

Plate 8.2. Dauer larvae present on the second abdominal tergite of *H. ater* adults (x 50)

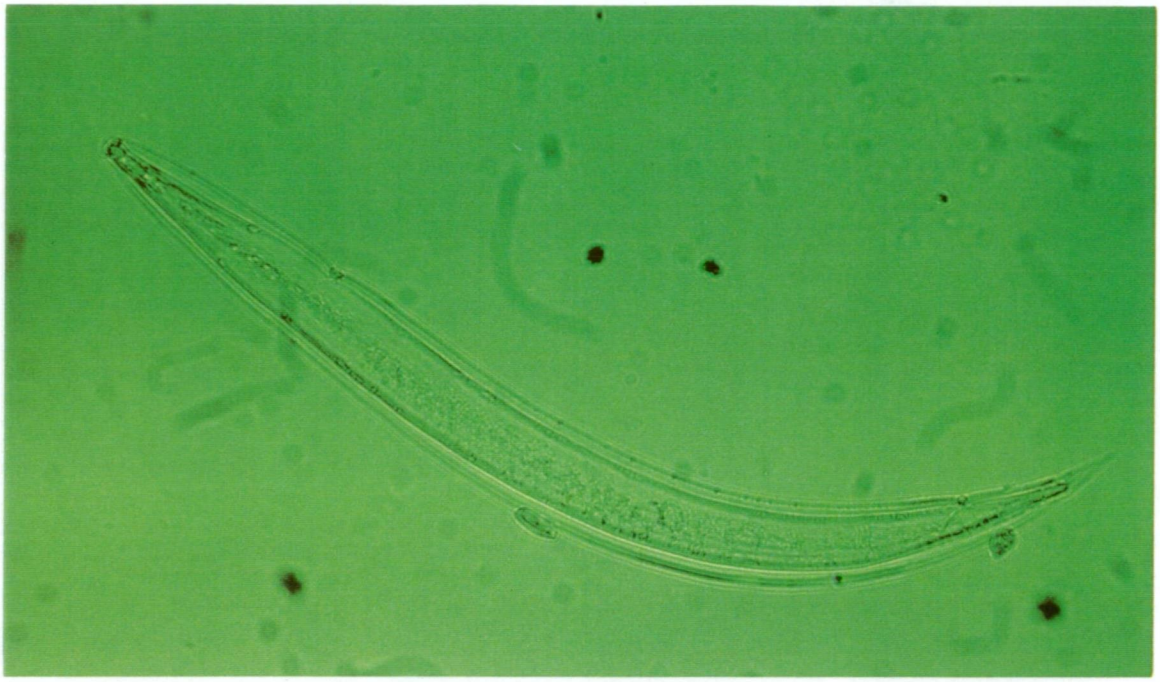

Plate 8.3. Dauer larva isolated from the second abdominal tergite of *H. ater* adults (x 400)

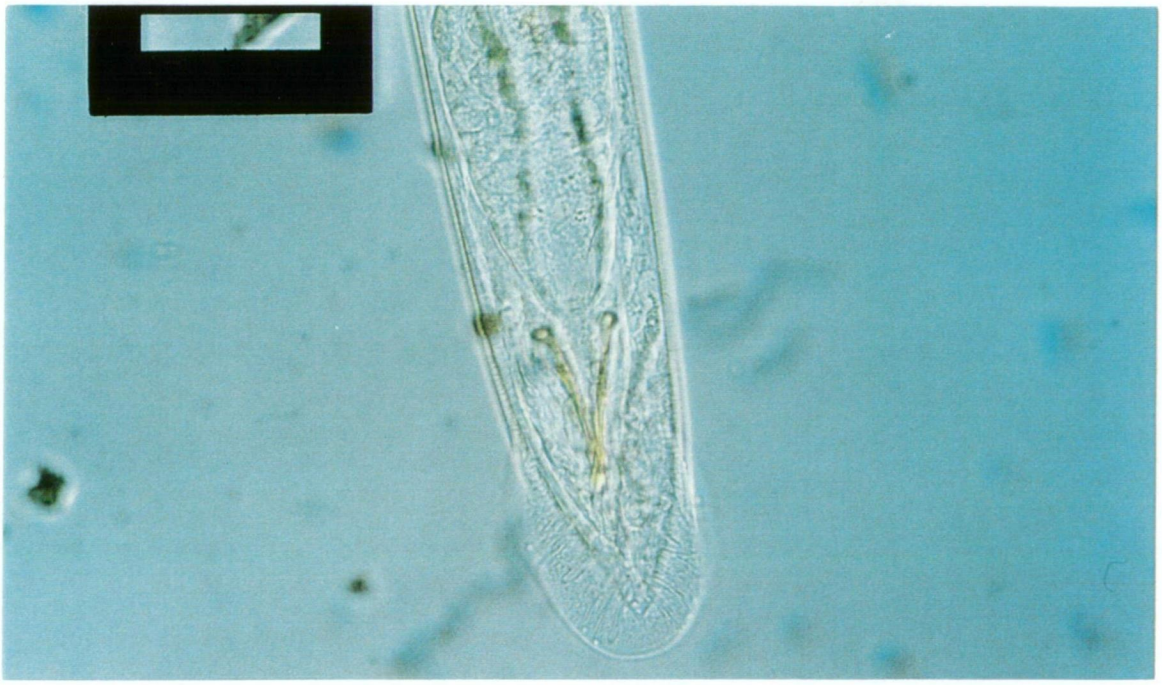

Plate 8.4. Male *Micoletzkya* spp. isolated from *H. ater* frass (x 1000)

CHAPTER 9 MANAGEMENT AND CONTROL

9.1. INTRODUCTION

The attack of *P. radiata* seedlings and young trees by *H. ater* in the Plenty Valley has been a significant problem. *H. ater* is capable of killing newly planted seedlings and up to 4-5 year old trees, while in the Northern Hemisphere the species has been known to kill mature trees 10 years old (Wulker 1923). The mortality of *P. radiata* seedlings in the Plenty Valley has resulted in costly replanting and delays of one to three years until final harvest.

Effective pest management requires a full understanding of the pests functions, its reactions to its environment and its biological and physical limiting factors. Therefore, an understanding of the life history and habits of *H. ater* was required to develop and evaluate alternative control methods while maintaining current management practices of slash conservation and reduced insecticide use.

Following the acquisition of data through field observation and experimentation for a range of aspects of the life history and ecology of *H. ater*, it was then appropriate to extend this information to the development of a risk assessment model. This was achieved by reference to and analysis of the existing seedling mortality/survival data formerly collected by ANM from 1990 to 1997 and relating this data to those factors influencing risk of attack in both space and time.

An overall management plan and risk assessment schedule was then formulated with respect to *H. ater* host plant selection and colonisation, its dispersal through the plantation, and various aspects relating to the attack of *P. radiata* material. Successful development and implementation of the proposed pest management program is expected to maximise seedling survival and reduce costs of production and protection.

9.2. LITERATURE REVIEW

9.2.1. Causes of Bark Beetle Outbreaks and Management

Outbreaks of bark beetle populations often occur suddenly and in combination with the abundant presence of suitable green unbarked slash, the existence of low vigour trees associated with drought, water logging, defoliation or senescence and climatic conditions that are warmer and drier than average (Neumann 1987). Bark beetle populations are capable of building up rapidly to epidemic levels with the availability of a large quantity of breeding material (Scott & King 1974).

Insects which affect plantation establishment and initial growth, such as *H. ater*, have both short and long term impacts (Hall & Gilbert 1989). In the short term, there is loss of initial investment and additional costs and effort associated with establishment of sites, and in the long term there exists the potential impact on the volume and timing of mature harvest (Hall & Gilbert 1989) and product quality (S. Hetherington, pers. comm.).

Effective management of bark beetles and other insects requires a knowledge of the elements which potentially regulate them, and is based on efficient detection, impact assessment and application of treatments where and when required (Garner & Harvey 1984, Hall & Gilbert 1989, Neumann & Marks 1990). Monitoring and detection enables the identification of problem areas and the build up of insect populations, so that decisions can be made based on previous impact assessment and appropriate treatments applied to minimise damage before outbreaks occur (Hall & Gilbert 1989).

Three methods of bark beetle management are described by Garner and Harvey (1984) which include doing nothing, direct control and indirect control. With the 'doing nothing tactic' one would expect similar levels of host mortality to continue in the future. Immediate mortality/eradication of the target organism is expected from direct control techniques, while indirect control techniques are not designed to cause immediate pest mortality but rather the maintenance of

either the bark beetle population or tree mortality below an acceptable level.

Neumann and Marks (1990) describe management practices in terms of direct, cultural, biological and genetic control. Neumann and Marks (1990) note for established exotic bark beetles, effective techniques are required for their long term management, rather than for eradication. Quarantine is an essential element of the overall pest management scheme for Australian pine plantations and a very important factor in excluding destructive agents from overseas (Ohmart 1980, Neumann 1987, Neumann & Marks 1990).

9.2.2. Direct Control Techniques for *H. ater*

9.2.2.1. Burning

Burning slash on harvested sites was the normal forestry practice prior to replanting which also destroyed any beetle life stages and made the slash unsuitable for breeding and feeding (Zondag 1982).

However, burning has disadvantages, and attack of fire damaged timber and stumps in New South Wales by *H. ater* has been observed (Neumann 1987). Boomsma and Adams (1943) described serious damage to seedlings following the burning of slash residue. *H. ater* was one of a number of bark beetles attracted by smoke from forest fires in Scots pine in Poland (Dominik & Litwiniak 1983).

9.2.2.2. Trapping methods of control

Trapping methods to reduce insect numbers have been utilised in Europe and other countries, and consist of both trap logs and billets baited with pheromone mimics.

9.2.2.2.1. Baited traps

The use of artificial bait traps has proven successful for many forestry pests assisting in monitoring regimes (Norlander 1989).

9.2.2.2.2. Log traps

Freshly cut pine logs that have been partially buried during times of beetle flight and left for some interval of time are lifted and destroyed. This method has been shown to be feasible in small areas but is very labour intensive and not practical where a large plantation size is involved (Clark 1932, Swan 1942). The number of trap logs required can be estimated using the formula derived by Tragadh and Butovitch (cited by Minko 1958) of L=AS(N-MN)/FT where L=number of trap logs, F=feeding area per trap log, A=smallest feeding area, N=number of beetles per acre, M=mortality before swarming, S=sex proportion and T=number of female specimens per tunnel. The use of trap logs (the number of which is determined using the formula) is described by Minko (1958), following the build up of a large beetle population over several years. However no practical information was given by this author as to the numbers of logs required or the effectiveness of the treatment method. Log traps are not used in Australia to any degree as a means of control (D. Boomsma, pers. comm.).

9.2.2.3. Biological control

The New Zealand Forest Service has undertaken investigations of the biological agencies that control *H. ater* under field conditions (Zondag 1982). In 1933, three species of *Rhizophagus* beetles were introduced from England and released but they have never been recovered (Miller & Clark 1935). In 1972, the New Zealand Forest Service commissioned the then CIBC (now IIBC) to initiate a search for biological control agents of *Hylastes* in Europe. The two pteromalid parasitoids *Rhopalicus tutela* Walker and *Dinotiscus eupterus* Walker were introduced into New Zealand from Eastern Austria in 1975. These two parasites attack *H. ater* larvae. Both *R. tutela* and *D. eupterus* were successfully reared and released in New Zealand. After two years, the success of establishment and the impact of the parasites on *H. ater* populations was deemed unsuccessful and breeding was discontinued. This lack of success was found to be due to the ovipositors of the parasites being unable to reach larvae developing under the bark

in contact with the soil or under bark thicker than 3 mm (Zondag 1979).

In 1976, the predatory clerid, *Thanasimus formicarius* L. was received from the CIBC station in Austria. Effectiveness of this predatory beetle was presumed to be high, as the predatory larvae have the ability to move under the bark in contact with the soil where the majority of bark beetles breed and the predatory adults can lay their eggs on any suitable material containing bark beetles irrespective of bark thickness. Some difficulties with the breeding of *T. formicarius* were encountered but overcome (Faulds 1988). *T. formicarius* is the only introduced biological control agent which has been recovered in New Zealand (Faulds 1989), however, its impact is doubtful as it is unable to effectively respond to the large populations of bark beetle experienced in New Zealand (Zondag 1979).

Studies on the prey specificity of the predator *Temnochila virescens* F. on *I. grandicollis*, also noted the acceptability of this predator to *H. ater* (Lawson & Morgan 1993).

In a review of the natural enemies of bark beetles in Europe, nine predator species of *H. ater* are listed (Mills 1983), although none of them prey specifically on the black pine bark beetle.

9.2.2.4. Preventive measures

9.2.2.4.1. Chemical sprays

In England, the Forestry Commission developed an insecticidal control by dipping seedlings before planting in a 1.6% Gammacol (γ-BHC) water solution (Scott & King 1974). They also found that spraying with 0.125% Lindane (γ-BHC) one to two years after planting, at a time when a heavy attack of beetles was anticipated or had occurred, provided effective control. Atkinson and Govender (1997) detail the effectiveness against *Hylastes* spp. of γ-BHC 0,6% dust.

Work by Heritage et al (1989) found that seedlings dipped in an insecticidal solution before planting sustained only 3% mortality compared to 19% mortality of untreated and control seedlings. Chemicals used were 1.6%

Lindane, 0.8% Permethrin, and 0.8% Cypermethrin. Ideally, dipped seedlings should provide protection for a complete season but would require additional sprays to maintain protection following a period of weathering (Heritage et al 1989, Atkinson & Govender 1997).

9.2.3. Indirect Control of *H. ater* through Forest Management Practices

The incidence of attack by insects on stumps, logs, logging slash, and ultimately seedlings, can be reduced by simple changes in management practices.

9.2.3.1. Change in length of rotation

Delaying second rotation planting for some extended period to enable full exploitation and exhaustion of breeding material, can prevent damage, as long as no harvesting of adjacent compartments has occurred in the meantime (Clark 1932, Swan 1942, Scott & King 1974). In South Australia, harvested *P. radiata* sites are currently left for at least one year before replanting occurs (D. Boomsma, pers. comm.).

Swan (1942) describes, under South Australian conditions that harvested areas may need to be left for two years before second rotation plantings are made. Scott and King (1974) describe *H. ater* as "characteristic of felling programs where annual felling areas, or coupes are not widely separated". Swan (1942) recorded *H. ater* as damaging young plantation *P. radiata* trees following a population pressure from breeding in nearby recently felled trees. Boomsma and Adams (1943) describe the increase in *H. ater* beetle numbers following clear felling operations.

There are also several disadvantages to this method including time delays, site deterioration and loss in yield (Clark 1932, Scott & King 1974, Cielsa 1988).

9.2.3.2. Improvement in forest hygiene

Appropriate hygiene is a major key to eliminating possible breeding sites of *H. ater* with the consequence of reducing population numbers to sub economic

levels (Cielsa 1988). Minko (1958) details the importance of good forest hygiene to enable "prevention rather than elimination".

Harvested logs should be removed as soon as possible from the logging site, with stock piles placed on skids to raise them off the ground (Zondag 1982). Any type of soil treatment such as ploughing, ripping or complete stump removal which promotes rapid drying of stumps and roots will limit the duration of the suitability of the breeding material and reduce population breeding sites (Clark 1932, Scott & King 1974).

In South Australia, logging residue on harvested sites is treated by chopper rolling immediately after harvest, which breaks the slash into small components (D. Boomsma, pers. comm.).

In some areas of second rotation pine in New Zealand, seedlings are not planted within one metre of an old stump to reduce the chance of attack (S. Hetherington, pers. comm.).

9.2.3.3. Promotion of healthy seedlings

Bark beetles are generally unable to kill healthy trees but will exploit physiologically weak hosts for their food supply (Rudinsky 1962).

Any technique, which promotes healthy seedlings, assists the plants ability to resist attack by the black pine bark beetle. Hadlington (1951), Minko (1962), Milligan (1978) and Zondag (1982) suggest that healthy vigorous seedlings survive attack by *H. ater*, with stressed (nutritionally or drought) and injured *P. radiata* plants being more readily attacked by *H. ater*. Improvement of growing conditions, the use of healthy, undamaged planting stock and careful planting technique to prevent damage will all assist the seedlings defences (Clark 1932, Milligan 1978).

9.2.3.4. Monitoring procedures

The monitoring of *H. ater* populations is an integral part of management, enabling early treatment and minimisation of damage (Boomsma & Adams 1943,

Alma 1975). Log traps provide an efficient method of trapping to monitor beetle numbers (Minko 1958). In New South Wales, where *I. grandicollis* is the major bark beetle species, pheromone traps are used to monitor beetle numbers (J. Moore, pers. comm.).

9.2.3.5. Stocking Rates

Within the Tasmanian forestry industry there is currently a theory that high stocking rates of trees (i.e. more than 1400 stems per ha) provides a large number of potential breeding sites if a clear felling regime is employed (S. Hetherington, pers. comm.). A change in management practices to a thinning regime may reduce stocking rates to 800 stems per ha after the first thinning and less than 400 stems per ha after a second thinning. It is expected that this thinning regime would not provide as many breeding sites at the clear felling stage.

9.3. MATERIALS AND METHODS

The dispersal of *H. ater* through the Plenty Valley plantation was traced by the study of records kept by ANM and my own investigations. The movement of *H. ater* from where the beetles were first detected in the original second rotation coupe to other harvested areas was followed for seven years. From this work the dispersal characteristics of *H. ater* were identified with respect to prevailing weather conditions, and how far the black pine bark beetle is capable of flying or being blown.

The ANM records were also used to determine if levels of seedling mortality of infested sites recorded in different areas were influenced by the time of harvesting, site treatment following harvesting and length of time between harvesting. In addition the potential effect of site preparation prior to planting, planting time, soil type, seedling type, site productivity, topography, site aspect and proximity to harvested and non-harvested areas were also examined with respect to *H. ater* movement through the plantation.

9.4. RESULTS

9.4.1. Tasmanian Situation

The *H. ater* problem was first noted following a change in forest management practice from the burning of harvested sites to leaving the slash on the soil surface in the interest of conservation of nutrients and soil structure. The bark beetles were potentially present in the plantation before 1990 but the problem was highlighted and accentuated with the large amounts of harvested slash resulting from the clear felling operations.

In the Plenty Valley, stocking rates of 600-1500 *P. radiata* trees/ha were utilised for the first pulpwood rotation. These coupes were clear felled with unprocessed logging residue remaining in the plantation. As shown in Table 9.1, the quantity of unprocessed logging residue (greater than 1.5 m in length) ranged from two to six m^3/ha. The total volume of slash remaining in the plantation is much higher, as assessments of unprocessed logging residue less than 1.5 m in length were not carried out.

Large numbers of *H. ater* beetles are attracted to *P. radiata* material in the order of over 200 beetles/m^2 of *P. radiata* material. Numbers of emerging beetles can be as high as 4000 beetles/m^2 of *P. radiata* material in contact with the soil. The combination of the large number of stumps (up to 1500 stumps/ha) which remained following harvesting and the volume of harvested slash, provided a huge host resource below and above the soil surface suitable for brood development.

In other mainland states lower stocking rates and thinning regimes are in operation so that the amount of logging residue at the final harvest is reduced. Markets also exist in other states to utilise logging residue for alternative products which assists in reducing the amount of harvested slash remaining in the plantation to a minimum.

Year of harvest	Ha	Unprocessed logging residue (weighted mean m^3/ha)
1989-90	172	1.94
1992-93	321	5.78
1993-94	253	3.53

Table 9.1. Unprocessed logging residue remaining following harvesting from coupes in the study site (>1.5 m wood type)

9.4.2. Movement of *H. ater*

Studying the movement of *H. ater* from where the beetles were first detected in the original second rotation coupe has shown that *H. ater* has dispersed throughout the entire Plenty Valley plantation to all harvested areas. The dispersal characteristics of *H. ater* follows one harvested area to the next proximal harvested area over a distance of seven kilometres. Records of infestation would suggest that beetles can disperse over two kilometres per year if wind assisted.

The prevailing winds in the Plenty Valley consist primarily of westerlies which would assist *H. ater* movement. The black pine bark beetle has been reported as a strong flier (Swan 1942) so would be capable of flying large distances but only if no suitable host material is available in the immediate vicinity. Adult beetles were never personally observed in flight but were noted landing on the windscreen of the vehicle of a forestry worker (B. Gordon, pers. comm.). It is assumed, due to the host habitat of *H. ater*, that beetles would not fly high above the ground in order to utilise host material within an area but would be capable of flying higher to avoid obstacles in their path or to non-adjacent coupes by winds in their search for suitable host material. Beetle movement may also be assisted over distance by logging debris on machinery and log transport.

9.4.3. Forest Management Practices

The following standard forest management practices were applied to most of the Plenty Valley plantation. Clear fell harvesting occurred all year round with site preparation for planting being conducted during January, February and March. This operation involved pushing slash into heaps, and then site cultivation by

ripping and mounding the soil. Approximately 25% of the harvested slash was left behind, and the slash heaps were burnt during April and May. Replanting occurred during the winter, primarily in July and August. The only exceptions to this routine were the various slash treatments previously mentioned in Chapter Four.

Due to a lack of appropriate data, the levels of damage recorded in different sites could not be related to the time of harvesting, length of time between harvesting and site preparation for planting, and the productivity of sites.

However, the location of damage sites in relation to harvested and non-harvested areas did influence seedling mortality. Second rotation areas of newly planted seedlings were nearly always adjacent to freshly harvested sites. The only exception was high seedling mortality being experienced in a few sites which had been left for two years before planting but harvesting was still conducted nearby.

H. ater is dispersed over the full range of soil types carrying plantations in the Plenty Valley. These soils primarily consist of imperfectly to well drained dolerite and mudstone derived forms with both sandy and clay loam top- and clay sub-soils. Some areas of high seedling mortality were associated with soil types which have reduced pine growth due to limited water and nutrient availability as a result of reduced rooting capacity, and were also prone to waterlogging.

Site topography and aspect did not appear to influence seedling mortality although four areas which recorded more than 40% seedling mortality were areas with steep slopes (>19%).

P. radiata seedlings were predominantly utilised in the second rotation plantation being planted at rates of 1250-1430 seedlings per ha. On some sites *P. radiata* cuttings were utilised. In a location 30 km north of the Plenty Valley where both seedlings and cuttings had been planted in the same coupe at the same time, the cuttings were preferentially attacked (B. Burns, pers. comm.). In this instance, the time interval between lifting and planting of cuttings was significantly longer because of delays in transport arrangements, and therefore the cuttings were possibly more stressed than the seedlings and thus more susceptible

to *H. ater* attack. Different genetics and nursery practices of the seedlings and cuttings may also have played a role in susceptibility to attack. Cuttings also have a larger surface area than seedlings and are perhaps more attractive.

9.4.4. Control Options

The various options which need to be considered by ANM for the management of *H. ater* are presented as direct and indirect control techniques and strategies with potential risk management schedules as summarised in Figure 9.1.

9.4.4.1. Direct control options

In general the **direct options** for control of *H. ater* include:

- burning slash, and the use of
- trapping methods,
- biological agents, and
- insecticides.

9.4.4.1.1. Burning

The traditional forestry practice of burning slash of harvested sites prior to replanting removed all logging residue and exposed stumps to fire. This practice destroyed all surface slash resulting in less substrate for *H. ater* attack. Thus reducing the number of potential breeding sites for *H. ater*.

The conventional burning practice conducted by ANM as described in Table 9.2, involved the removal of at least 70 to 100% of the logging residue. This practice was conducted with a bulldozer pushing logging slash into windrows which were subsequently burnt two to three months later. The sites were then strip cultivated to prepare for planting by the re-alignment, ripping and mounding of the planting rows. Although cost effective, the practice is not environmentally sustainable due to the loss of nutrients and site deterioration associated with burning.

9.4.4.1.2. Trapping

Throughout the duration of the study the use of felled and semi-buried *P. radiata* billets as lures for ovipositing beetles proved successful in all investigations. The use of major monoterpenes notably α-pinene, and commercially available primary bark beetle pheromones, attractants and repellents when used in combination with *P. radiata* billets did not increase catch numbers significantly. In addition, the use of commercially available traps and methods which are employed chiefly for primary bark beetles were shown to be ineffective compared to the use of *P. radiata* log traps (See Chapter Two).

The use of log traps as a suppression method as suggested by Clark (1932), Swan (1942) and Minko (1958) was investigated to determine its potential under Tasmanian conditions. Operationally, it was assumed that the total surface area of the trap billets had to be of the same order as the surface area of the logging slash and stumps (i.e. the amount of attractive material on the harvested coupe). It was found that the suggested method of placing trap billets around the edge of a coupe (or in one corner of a coupe) was neither economically nor practically feasible (See Appendix 24 for calculations and assumptions). Following computation, the number of trees required per ha to act as a barrier was often greater than the number of planted trees per ha. Even if all slash is removed, or chopped into small pieces which are not attractive to the beetle, the number of trees required for billet production to compete with the remaining stumps was still 300-700 per ha. This is still too high to be a viable alternative method. Inquiries that have been made in South Australia, New South Wales and Victoria indicate that the use of log traps has never been adopted operationally for the suppression of *H. ater* (D. Boomsma, pers. comm., J. Moore, pers. comm., P. Whiteman, pers. comm.).

9.4.4.1.3. Biological agents

Attempts at biological control in New Zealand have been unsuccessful due primarily to the inability of the predators and parasites of *H. ater* life stages to reach the large numbers in the bark in contact with the ground. None of the

predators of *H. ater* in Europe listed by Mills (1983) prey specifically on *H. ater* so biological control would appear very difficult if such a long term method was attempted in Australia.

The use of pathogens is also not viable due to the limiting factors associated with the soil environment, with temperature playing a significant role in limiting the invasiveness of entomopathogenic fungi. The truly parasitic nematodes found in association with *H. ater* adults do not appear to be effective in reducing beetle numbers (See Chapter Eight).

9.4.4.1.4. Insecticides

The intensive use of insecticides as a preventive measure is neither environmentally defensible, sustainable, nor effective. The use of Cypermethrin as a dipping treatment prior to planting (as detailed by Scott & King 1974, Heritage et al 1989) may be of short term use where high beetle populations exist. However, protection would need to be maintained with follow up sprays. The use of Cypermethrin as detailed by Scott and King (1974) applied by knapsack in the field would cost approximately A$ 62 per ha (S. Hetherington, pers. comm.).

The use of *P. radiata* billets to detect high beetle population sites would permit insecticidal dipping of seedlings prior to planting. Forest supervisors would oversee such an operation and make the decision to apply insecticide to planting stock on the basis of the analysis of data collated by field workers.

The associated environmental problems of insecticide use including longevity of the chemical and application hazards for field workers are important factors to be considered in plantation management. ANM's policy is to minimise and restrict insecticide use to those occasions when the use of other control techniques is not possible.

9.4.4.2. Indirect control options

Indirect control options involve changes in management practices to reduce the incidence of *H. ater* attack on stumps, logs, logging slash and

ultimately seedlings and include

- Delaying second rotation plantings
- Slash and stump treatment
- Harvesting programs
- Monitoring
- Promotion of healthy seedlings

9.4.4.2.1. Delaying second rotation plantings

Delaying second rotation plantings to enable full exploitation and exhaustion of breeding material, can prevent subsequent damage. This practice has been successfully used in some mainland Australian states and is effective as long as no harvesting has occurred nearby in the meantime (D. Boomsma, pers. comm.). To be successful in Tasmania harvested areas would have to be left at least 12 months for the utilisation of the majority of harvested slash and up to 2-3 years to enable exhaustion of large diameter slash and stumps before replanting can occur. The rotation length of *P. radiata* sourced by ANM for use in paper manufacture is only 15-18 years. This does not allow for a delay between harvesting and replanting in situations where the planting program cannot be maintained. In the Plenty Valley most site specific harvesting and replanting was achieved within one year with a fallow period of only eight months, due to the lack of availability of other suitable sites to maintain the planting program.

The disadvantage of delaying second rotation plantings results in an extended rotation length which may not be feasible on those occasions when seedling mortality levels are low. Additional problems are encountered when the fallow period is more than one year and these may include site erosion, weeds and visual impact problems that need to be addressed before replanting.

When seedling mortality is high, the cost of replanting and time delays on fibre production would be greater than the cost of delaying the re-establishment of the plantation. Under these circumstances, delaying second rotation plantings would be economically viable.

9.4.4.2.2. Slash and stump treatment

The alternative slash management techniques to enable slash retention with currently available machinery are outlined in Table 9.2 as treatments B, C, D, E and F respectively. All treatments involve the spreading of the remaining logging residue evenly over the site followed by either spot or strip cultivation in preparation for replanting. The treatment of logging residue needs to immediately follow harvesting or at least within ten weeks to prevent complete beetle development (See Chapter Three).

Treatment B involves the use of an excavator to spread the logging residue evenly over the surface which is then followed by spot cultivation with the use of a Rotree® spot cultivator unit fitted to an excavator.

Treatment C involves the spreading of logging residue with a bulldozer followed by spot cultivation with the use of a Rotree® spot cultivator unit attached to an excavator.

Treatment D involves the logging residue being chipped with a drum chipper and being spread over the site followed by the use of a bulldozer for strip cultivation with the realignment of windrows, ripping and mounding of the planting rows.

The fourth and fifth alternative treatments E and F both involve the use of a chopper roller to chop the slash as it moves over the coupe area. This operation involves two rollings, one following harvesting and a second lighter rolling after slash has dried. The chopper rolling is followed by spot cultivation for treatment E by the use of a Rotree® spot cultivator unit fitted to an excavator, and strip cultivation for treatment F by the use of a bulldozer as previously described. Currently, however, chopper rolling machinery is not available in Tasmania.

As shown in Table 9.2, treatment B is the most expensive slash management technique costing A$ 1300/ha, while treatments C, D and E have similar costings of A$ 950/ha, A$ 920/ha and A$ 890/ha respectively, while treatment F is the most cost effective method at A$ 390/ha.

Treatment	Description	A$/ha
A	Conventional Burning with 70% Slash Removal and Strip Cultivation	490
B	Slash Retention (Excavator spreading) with Spot Cultivation	1300
C	Slash Retention (Bulldozer spreading) with Spot Cultivation	950
D	Slash Retention (Slash chipped with a drum chipper) with Strip Cultivation	920
E	Slash Retention (Slash chopped with a chopper roller) with Spot Cultivation	890
F	Slash Retention (Slash chopped with a chopper roller) with Strip Cultivation	390

Table 9.2. Cost benefit of alternative slash management techniques

In the future treatment of stumps could be combined with treatment of slash to effectively eliminate breeding sites. The stumps could be destroyed at the time of harvesting by mulching *in situ*. This could be achieved by the use of machinery such as the T275 Hydra Stumper®. However, this machinery is currently unavailable in Australia. This technology would significantly increase the cost of treatment as the machinery costs in excess of A$ 1500/ha.

Current harvesting machinery which cut trees as low to the ground as possible could be recommended in order to minimise the height of the stump above the surface of the soil which results in a reduction in the number of beetles attracted (as shown in Chapter Four).

Alternatively, the slashing of stumps and logging slash could be considered at a cost of approximately A$ 500/ha. The slashing of stumps would reduce the height of the stumps to level with the soil surface.

The complete burial of stumps and slash is an option as *H. ater* is unable to attack host material that is completely buried at least 20 cm below the soil surface (See Chapter Four). This, however, is not a practical solution as the costs involved would be prohibitive.

9.4.4.2.3. Harvesting programs

It is recommended that any harvesting program should eliminate the clear felling of large adjacent coupes. This would ensure that harvesting and replanting operations occur at some distance from each other. Ideally, harvesting and replanting operations would need to occur at opposite ends of a plantation. The program would rotate over time (e.g. north vs south, east vs west, south vs north and west vs east). Current observations indicate that the distance between operations should be no less than two kilometres, with the use of monitoring trap logs in the vicinity of both treated areas to monitor beetle activity. Minko (1962) describes the spread of *H. ater* by expansion of the local population rather than migration from distant areas.

Adoption of the current thinning practice in the remaining coupes in the Plenty Valley will prevent the creation of the same set of circumstances which resulted from the clear felling operations of highly stocked stands. The levels of logging residue, as shown in Table 9.1 would be greatly reduced and levels of seedling mortality should be reduced. It is also recommended that thinning operations should not occur adjacent to newly planted coupes.

9.4.4.2.4. Monitoring

Monitoring is the most important activity to enable the detection and localisation of outbreaks. The use of semi-buried billets has been shown to be a useful tool in mapping beetle incidence and identifying 'hotspots'. Billets should be established at the time of harvesting and maintained through the establishment phase. The billet size would have to be of manageable proportion to enable establishment in contact with the soil and ease of removal by individual field workers. Billets with larger bark diameters and higher moisture contents would maintain their effectiveness longer. All billets should be monitored on at least a monthly basis with their effectiveness maintained within three months. Monitoring for presence/absence would be performed by field staff who would relay information to their supervisor. In consideration with the forest manager the

supervisor would make decisions and recommendations based on costs and risks.

9.4.4.2.5. Promotion of healthy seedlings

H. ater attack of living trees is primarily restricted to moribund or stressed trees. Tree stress may arise through defoliation, drought, excess rainfall or flooding, mechanical injury, fire competition, diseases and herbicides. Improvement of the growing conditions, the use of healthy, undamaged planting stock and careful planting, will all contribute to good seedling establishment. Practices which maintain good seedling growth should be incorporated into the planting process to minimise any stress placed on seedlings. Practical measures including good nursery techniques (such as good mycorrrhizal infection of seedlings, minimisation of topping seedlings especially within eight weeks prior to lifting, and good nursery nutrition and hygiene), reducing the time period from lifting to planting, careful handling of seedlings during transport, machine planting or the use of containerised seedlings in beetle prone areas may be utilised to reduce stress levels.

Timing the planting of seedlings to avoid the times during the year when the majority of adult beetles emerge may assist seedling survival. Currently planting usually occurs during the months of July and August but if planting occurred early in May, while there is still warmth in the soil, seedlings would have more chance of establishing before beetle emergence in August. However, the study area is often too dry in May to enable early planting. In addition, adult beetles are present all year round so there exists the potential for attack of seedlings planted in any month of the year if temperatures are favourable.

9.4.4.2.6. Record keeping

Keeping accurate records of remaining logging residues could enable the identification of areas that may be more susceptible to *H. ater* invasion so that preventative measures could be taken. Again keeping more accurate records concerning the time of harvesting, site treatment following harvesting, length of

time between harvesting and site preparation prior to planting would assist in the overall understanding of *H. ater*, and hence its better management over time.

9.4.4.2.7. Competitive exclusion

The competitive exclusion of *H. ater* from stumps by the encouragement of the present secondary, non damaging scolytid *H. ligniperda* was suggested (P. Volker, pers. comm.). This is unlikely to be successful because if *H. ligniperda* was capable of excluding *H. ater* one would expect that it would be doing so without any intervention. *H. ater* is consistently the dominant species most often found in *P. radiata* slash and stumps. Only on a few occasions was *H. ligniperda* found to be coexisting with *H. ater* in equal or higher numbers.

9.5. DISCUSSION

The situation in Tasmania differs to other mainland states in terms of the quantity of logging residue that remains following harvesting procedures. Firstly, in the Plenty Valley, higher stocking rates existed for the first pulpwood rotation and sites were clear felled without any previous thinning, so the level of logging residue was considerably higher than in mainland states where lower stocking rates existed and thinning regimes were employed. Secondly, in Tasmania there were no down stream markets to utilise *P. radiata* material unsuitable for pulping and therefore such material remained in the plantation.

The burning of all harvested slash prior to the planting of seedlings has been the traditional management practice in second rotation *P. radiata* plantations. This practice removed the slash, thus reducing or eliminating *H. ater* host material and limiting developing broods. The original management program involving the exposure of stumps and removal of slash by the burning of harvested sites, although cost effective, is not environmentally sustainable and is no longer an option due to the change in field management practice to one of leaving the slash on the soil surface in the interests of conservation of elements and soil structure.

The change in forest management practice to one of slash retention

provided the beetles with a large source of suitable *P. radiata* host material. The adopted harvesting program involving the clear felling of large coupes adjacent to planting areas provided beetles with a continuous supply of suitable breeding material. The essentially continuous breeding of *H. ater* with adult beetles attacking host material at most times throughout the year enables the exploitation of all available suitable material. This combination of variables together with favourable weather conditions provided optimal conditions for the proliferation of the *H. ater* population and the resultant mortality of *P. radiata* seedlings.

The management options available to ANM presented in the form of direct and indirect control techniques are summarised in Figure 9.1. These options must be considered in light of the Tasmanian situation and the current forest management practice of slash retention.

Under the existing management the ultimate solution to the *Hylastes* problem would involve the complete removal of the remaining stumps and slash following the harvesting procedures. Stumps are the primary source of brood population, although the slash that is either embedded or in contact with the soil also contributes suitable breeding sites. Removal of or treating potential host material would destroy *H. ater* breeding sites. The practicality and cost of such a procedure in large scale plantations, combined with the resulting problems of site deterioration and nutrient loss, would appear to be too high.

Modification of the beetle habitat would provide options for control by reducing the number of breeding sites. The two alternative slash management techniques described as treatments B and C in Table 9.2, both involve the spreading of logging residue (with an excavator for treatment B and a bulldozer for treatment C) followed by spot cultivation with a Rotree® unit attached to an excavator. The use of an excavator to spread logging residue in treatment B enables slash to be spread more evenly than a bulldozer, with minimal site impact, although the cost of using an excavator is approximately twice that of a bulldozer. The Rotree® unit cultivates one metre diameter circular spots within the slash to enable replanting. The Rotree® unit for spot cultivation enables access to steep

sites (>19% slope) and has minimal site impact with less soil disturbance and compaction. Increased planting costs are encountered with both treatments B and C due to the large quantities of coarse logging residue spread over the surface, making planting difficult and dangerous. These two slash retention techniques still enable *H. ater* brood development by maintaining the bark-soil interface of slash components.

The two slash retention techniques, E and F involve the use of a chopper roller. The chopper rolling treatment involves the use of large rollers to break logging residue into small components. In order to breakdown the slash sufficiently, two separate chopper rolling operations would be required. Ideally the first operation would be conducted within ten weeks of harvest to accelerate the drying of slash and minimise beetle brood development. The second operation using a lighter roller would be carried out after the slash had dried out sufficiently to enable more efficient chopping of the slash. Treatment E is followed with spot cultivation by the use of a Rotree® unit attached to an excavator, and treatment F with strip cultivation by a bulldozer, as previously described. Treatment F was the most cost effective method of treating the slash. Although, the major disadvantage with the chopper rolling technique is the embedding of slash items in the soil, and therefore maintenance of the bark-soil interface required for *H. ater* development. Other disadvantages include soil compaction associated with three passes of heavy machinery over the site.

Mulching of logging residues into small pieces to ensure that the problem was not confounded was found to be the most effective method of treating the slash. The removal of bark from slash would remove the bark-soil interface that is required by *H. ater* for feeding and ultimately brood development. The third slash retention treatment D involving the logging residue being chipped with a drum chipper over the site followed by the use of a bulldozer for strip cultivation with the realignment of windrows, is the most effective slash retention option for beetle control. This treatment breaks logging residue into small parts enabling easier planting with strip cultivation. This technique eliminates the number of potential

breeding sites due to chipping of slash pieces. It removes the bark-soil interface and the smaller pieces dry out more rapidly than the large pieces remaining under treatments B, C, E and F. The treatment of logging residue needs to immediately follow harvesting to prevent beetle invasion and at least within ten weeks to prevent complete beetle development.

Treatment of slash could be combined with treatment of stumps to effectively minimise breeding sites. The available machinery to destroy stumps by mulching *in situ* are expensive and not cost effective, but may be considered if seedling mortality remained high. A Hydra Stumper® is capable of removing remaining stumps up to 165 cm above and 109 cm below the soil surface (Anonymous 1998). This machinery may be used in the future but is currently unavailable in Australia. The harvesting of stumps as low as possible to or below the soil surface would also be recommended to reduce their attractiveness.

Adoption of a thinning regime is recommended to minimise the quantity of slash at each harvest and ultimately at the final harvest. The clear felling of adjacent coupes needs to be minimised. If a clear felling regime is necessary then harvesting and replanting operations need to occur at some distance (>2 km) from each other.

Monitoring of beetle populations is the most important activity in the overall management of *H. ater*, with the use of *P. radiata* billets being the most effective method in mapping beetle incidence in time and space. Monitoring detects the presence of a beetle population and its fluctuations over time. Forest management decisions concerning the application of direct and indirect control techniques can all be based on data collated through monitoring procedures. However, more detailed studies are required to determine economic thresholds, for instance providing a range of beetle numbers that would indicate when control measures needed to be implemented.

The use of insecticides although not environmentally sustainable, may be considered by the forest manager in newly planted areas to prevent damage by destroying invading adult beetles where monitoring has shown the presence of

high beetle numbers. Where high beetle populations exist, insecticide use as a seedling dip may also be considered in an attempt to repel beetle attack. The environmental side effects of insecticide use and the safety aspects for field workers need to be considered so insecticide use is restricted to those occasions when no other control techniques are possible.

The need to quickly revegetate sites after harvesting places pressure on the forest manager to re-establish sites quickly, so delaying replanting is not a favoured option. Delaying second rotation plantings for at least 12 months up to three years may be considered by the forest manager in areas of high beetle population where high seedling mortality could be expected.

Although the effective use of trap logs for monitoring purposes has been demonstrated it has been shown that the use of trap logs would not be an effective suppression method. As detailed in Appendix 24, the required number of semi-buried billets arranged in furrows around susceptible coupes to act as an effective barrier is too high. The cost of such a procedure and the time involved to establish and then subsequently destroy billets is uneconomical when all costs (including the cost of the billets and labour costs of establishment and removal) are considered.

Field studies have shown that the use of traps baited with major monoterpene hydrocarbons have no advantage over the use of *P. radiata* billets alone in capturing *H. ater* beetles. Similarly, it was shown that the presence of a range of materials involved in host selection and colonisation of primary bark beetles, did not affect beetle catch numbers on *P. radiata* billets. The effectiveness of *P. radiata* billets in capturing *H. ater* adult beetles compared to the various trapping methods presented in the literature chiefly for primary bark beetles was also demonstrated. Although the use of semi-buried billets has been shown to be impractical as a means of suppressing beetle population they do play an important role in monitoring population occurrence and density.

The use of biological agencies including entomopathogenic fungi and nematodes has been shown to be impractical due primarily to the subterranean

habitat occupied by *H. ater*.

Any technique which promotes healthy seedlings, assists the plants ability to resist attack by the black pine bark beetle. Careful handling and planting of seedlings and minimising the time between lifting and planting are basic techniques that could be incorporated into a planting regime which will ultimately reduce seedling stress, and assist plant establishment. Containerised seedlings could be considered in beetle prone areas.

The importance of the need for accurate record keeping was highlighted as a result of this study. The compilation of accurate information concerning site description, time of harvesting, harvesting procedures, processed and unprocessed *P. radiata* material, site treatments following harvesting, length of time between harvesting and site preparation prior to planting, planting stock, and seedling mortality would all contribute to a better understanding in the future management of *H. ater*.

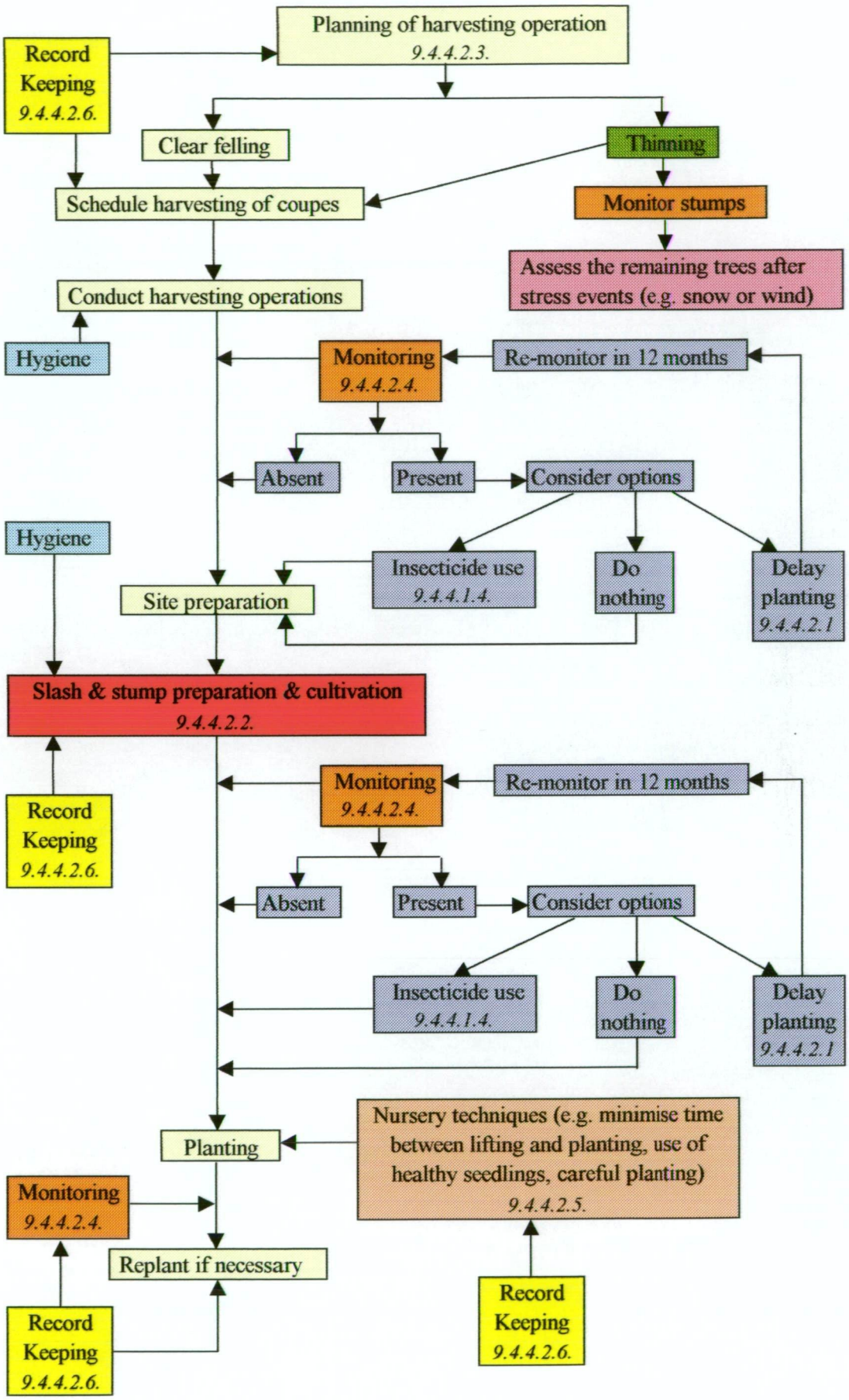

Figure 9.1. Options for management of *H. ater* (Numbers correspond to thesis sections)

CHAPTER 10 GENERAL DISCUSSION

10.1. SUMMARY

The primary objective of this study was to gain an understanding of the black pine bark beetle, *H. ater* under Tasmanian conditions to assist in the development of an effective, economic and environmentally sustainable pest management program in order to minimise the current significant losses of seedling replants in second rotation *P. radiata* plantations. Important aspects of this study included the documentation of *H. ater* biology and ecology, physical, chemical and microbial aspects of host selection and attraction, and relationships with microorganisms.

While the results presented in each chapter have been previously discussed the aim of this general discussion is to bring together the relevant findings and discuss the interrelationship of the numerous factors influencing *H. ater* with regard to past and current management practices. Based on this integration recommendations are made for reducing the future pest status of *H. ater*.

From the outset the unique and below ground habitat utilised by *H. ater* posed some difficulty to investigations with the involvement of the additional dimension of the soil environment. Numerous trapping devices utilised in the Northern Hemisphere in research and trapping programs chiefly for primary bark beetles (Chapman & Kinghorn 1955, Browne 1978, Furniss 1980, Lindgren 1983) were ineffective when trialed against *H. ater*. The use of billets cut from 17-20 year old *P. radiata* trees was the most successful and efficient method for catching *H. ater* beetles. The use of billets proved successful in monitoring beetle numbers and enabled easy access to life stages compared with the harvesting of stumps. The difficulty of assaying potential chemical attractants separate to host material was demonstrated.

H. ater was discovered in the Plenty Valley Tasmania in 1990 and the secondary species *H. ligniperda* in 1993. Under Tasmanian conditions *H. ater* always predominates over *H. ligniperda* which differs from other mainland states

where *H. ligniperda* usually predominates over *H. ater* when both species coexist (Neumann 1979, 1987).

Adult *H. ater* beetles invade *P. radiata* billets established in any month of the year as adults are present all year round, although beetle numbers are the highest during November-January, March-April, and August-September when (mature) adults emerge. Following emergence the feeding by adults causes extensive damage to seedling trees. Levels of seedling mortality recorded in the Plenty Valley of up to 90% are higher than recorded information from other mainland states (Swan 1942, Boomsma & Adams 1943, Minko 1961b). The higher levels of seedling mortality recorded in Tasmania, would primarily be due to the large amount of logging residue which remained in the absence of fire following harvesting procedures and hence suitable *H. ater* breeding sites. The cooler climatic conditions in Tasmania compared to other mainland states would also play a role in maintaining suitable moisture content in host material. In Tasmania, *H. ater* is solely responsible for seedling attack whereas in mainland states both *H. ligniperda* and *H. ater* have caused large numbers of seedling deaths (Neumann 1987). The presence of three distinct *H. ater* generations during the year is the same as experienced in other mainland states (Minko 1961a, 1962) and in New Zealand (Clark 1932).

No one sex is responsible for initial host selection with both male and female adult *H. ater* beetles arriving simultaneously to fresh *P. radiata* material in equal numbers within the first week of placement. The female initiates substrate entry through the bark into the phloem region and begins excavation of an egg tunnel with the male following behind, an event which was also found by Clark (1932) and Milligan (1978). A characteristic niche is formed at the beginning of the gallery which may be utilised as a nuptial chamber for mating or as a turning niche. As mating was observed prior to and during entry into the host and within the gallery this suggests that the characteristic niche acts as a turning niche with *H. ater* being a monogamous species. Stark (1982) describes that for monogamous bark beetle species mating may occur on the bark surface near the

entry hole or in the tunnel in a turning niche while polygamous species mate solely in a specialised nuptial chamber. The presence of hairs on the last abdominal sternite of male beetles which is lacking in females is a useful character for sex differentiation of adult *H. ater* beetles.

Once the eggs hatch the larvae progress through four larval growth stages or instars as documented also in New Zealand (Milligan 1978). In the studies reported here it was found that the total brood period for *H. ater* from initial invasion through to adult beetles takes 70 days (ten weeks) at a constant temperature of 25°C through to 115 days (16 weeks) at 15°C. The development of *H. ater* is proportional to temperature with the rate of development increasing with temperature although maintenance of appropriate moisture conditions would inhibit successful brood development at temperatures higher than 25°C.

In the Plenty Valley, adult *H. ater* beetles attack stumps, logs and logging slash remaining after both clear felling and thinning operations as found by Clark (1932), Swan (1942), Boomsma and Adams (1943) and Hadlington (1951). *H. ater* only attacks host *P. radiata* material which is in direct contact with the soil as also detailed by Clark (1932), Bain (1977), Milligan (1978), Zondag (1982), Eldridge (1983) and Neumann (1987). *H. ater* is capable of attacking host material at depths of more than 80 cm below the soil surface, so there is an abundant host resource below the ground made up of all the stumps and root systems remaining after harvesting. *H. ater* will not enter *P. radiata* material which is completely buried below the soil surface, but requires some of the host material to protrude above the soil surface even if only slightly. The majority of the stumps and logging residue remaining after harvesting operations are utilised by *H. ater* as breeding sites with a large subterranean host resource and material lying on the soil surface enabling large populations to build up rapidly. *H. ater* will not attack other indigenous and/or exotic tree species, only pine. *Acacia* and *Eucalyptus* spp. wrapped in *P. radiata* bark will be entered by *H. ater* but then rejected after contacting the bark-wood region of the billet.

H. ater feeds on bark of any thickness and is able to successfully complete its entire life cycle within bark which is only 2 mm wide. So there are a large number of potential feeding/breeding sites as long as the moisture content is suitable, and there is direct contact with the soil. The slash with thinner bark is attacked first and often acts mainly as a food source for beetles, but is capable of supporting a developing brood through to maturation if conditions are suitable. Swan (1942) describes breeding of *H. ater* in thick bark although Scott and King (1974) detail the ability of *H. ater* to breed in small sized material less than 10 cm in diameter. The importance of temperature and indirectly moisture content on regulating the successful utilisation of thin bark for brood development was demonstrated in the laboratory temperature trials. At high temperatures of 25°C successful life cycle development was restricted to thicker barked portions of the billets.

Moisture content plays an important role in determining the suitability of the host material. Dry *P. radiata* material is not suitable or selected by *H. ater* but it has the potential to become attractive once its moisture content is increased. This was demonstrated by resoaking unattractive dry billets and then finding them to be invaded by *H. ater* following establishment in the field.

Results from extensive chemical analyses of feeding adult beetles, host tree material and field trials of primary bark beetle attractants indicate that *H. ater* utilises *P. radiata* host monoterpenes as the primary attractant in the host selection and attraction process with no one compound acting in isolation but a combination of host monoterpene compounds. The dominance of α-pinene in all successfully attacked material and from the results of the olfactometer assays would suggest its single significance. Damaged or freshly harvested *P. radiata* material contain a number of monoterpenes in high quantities and over time the general level of host compounds decline. *H. ater* are able to differentiate suitable host material and that which is unsuitable by the level of host compounds released by *P. radiata* while the resinosis associated with lesions of living trees would act to direct attack to moribund/weak trees.

Both stumps and harvesting residues are utilised by *H. ater* primarily within the first year after harvesting but stumps are capable of supporting developing broods for two years. Moisture content combined with soil contact are the primary determinants of host suitability for *H. ater* attack. Stumps and logging residues become unattractive to *H. ater* over time corresponding to a decline in the level of host volatiles that are released to act as primary attractants and the corresponding moisture content decline being unsuitable for brood development.

Beetle-produced compounds are not utilised by *H. ater* in the initial host selection process. The attack by *H. ater* of stumps, logging residues and seedlings does not require a mechanism for overcoming host resistance such as primary bark beetle species which attack living trees. Primary bark beetle species such as *Dendroctonus* which attack healthy mature living trees utilise insect produced compounds in association with host produced compounds to cause a mass aggregation of beetles on the host tree to overcome host resistance and ensure successful colonisation. The release of semiochemicals above the ground provides a successful means of communication which would be inhibited within the soil environment. The increase in the unknown compound and oxidised monoterpenes detected in *H. ater* beetles following several days of feeding after the majority of beetles have arrived at host material could play a role in signifying host occupancy to other beetles. They have no effect on primary host selection and attraction. Thus, primary attraction appears to be mediated by a complex of compounds dominated by α-pinene while semiochemicals associated with typical primary bark beetles are either absent or in trace amounts. An examination of the potential involvement of microorganisms in the host bark-sapwood interface in primary attraction would be recommended in any future work. The field and laboratory response of beetles to MCOL and a combination of *cis*-verbenol and MCOL might indicate their possible involvement. This response may reflect a former dependence on such materials which has been obscured by the adaptation

to the subterranean habitat. However, it did show that the development of a management program based on secondary attraction would not be possible.

The ability to produce sound by male *H. ater* beetles would appear to be of assistance once beetles have arrived at the host material. Due to both sexes arriving simultaneously and existing within the soil, sound would not be of assistance in initial host selection and attraction, but would appear to be of use within the host in courtship and mate pairing or defence of the female and developing egg gallery.

A number of microorganisms were commonly found in association with *H. ater* including ten bacterial species, two yeasts and the fungus *L. lundbergii*. The microbial isolates from *H. ater* were not shown to be involved in host selection and attraction of *H. ater*. Fungal spores were observed by SEM to be concentrated in surface pits on the head, pronotum and elytra of both male and female adult *H. ater* beetles. *H. ater* must encounter a range of microorganisms within the soil habitat, but the constant association of similar microorganisms found in the beetles but not in unattacked material, in particular, *L. lundbergii*, strongly suggests an active functional role. The presence of the fungal isolate in *H. ater* galleries indicates that it could play a role in host colonisation by making the host suitable for brood development and in nutrition. The importance of microorganisms within the Scolytidae in conditioning the host as a suitable breeding site and provision of a food supply has been demonstrated (Whitney 1982, Beaver 1989).

Similar nematodes were found in association with *H. ater* in Tasmania as detailed by Dale (1967) in New Zealand and Ruhm (1956) in Germany. The *Neoparasitylenchus* species isolated from *H. ater* appears to be a true parasite in that it does not kill its host, being of limited use in natural control.

10.2. GENERAL RECOMMENDATIONS

Practically, the findings of this study indicate that

a) low survival of *P. radiata* seedlings resulted from a number of essentially poor management practices which created ideal conditions for *H. ater* attack and development and,

b) more attention to monitoring and silvicultural procedures are required to minimise the significance of *H. ater* attack.

The control and management of *H. ater* must be based on reducing the incidence of attack on stumps, logging slash and seedlings, by changes in forest management practices based on a better understanding of the beetles behaviour and biology.

The ultimate solution would involve the complete removal of all remaining stumps and slash following harvesting procedures to eliminate potential breeding sites, and hence population numbers. As burning is not environmentally sustainable, the practicality and cost of such a harvesting schedule would be too high and could result in further site deterioration and nutrient loss.

An essential key factor in preventing *H. ater* development is removal of the bark-soil interface. The removal of the bark *in situ* would provide a viable method to prevent beetle attack. To enable the current practice of slash conservation, the chipping of harvested residues provides the most efficient method in minimising the number of potential breeding sites although the stumps would still enable beetle development. The number of slash items embedded in the soil need to be minimised to enable more rapid drying of slash on the soil surface, so the chipping option would be recommended rather than the chopper rolling method which would embed slash items in the soil.

Delaying the planting of the second rotation for some period (at least 12 months up to 2-3 years) to enable full exploitation and exhaustion of breeding material, can prevent damage as long as no harvesting in the immediate area has occurred in the mean time. This technique would be economically viable when seedling mortality is high as the costs of replanting and time delays on fibre

production would be greater than the cost of delaying the re-establishment of the plantation.

The planting of seedlings remote from newly harvested areas or areas planned for current harvesting would ensure containment and minimise outbreaks. Highly stocked stands should not be clear felled.

The implementation of a monitoring program is essential to track beetle numbers and identify areas which may be susceptible to attack so that the planting of seedlings could be delayed in such areas or other treatments applied. Accurate record keeping of all harvested sites should also be integrated into the overall management program.

10.3. FUTURE WORK

The involvement of *P. radiata* host compounds, other commercial semiochemicals and microorganisms associated with *H. ater* in the host selection and attraction process of *H. ater* was evaluated during this study. However, the role of microorganisms in the *P. radiata* bark sapwood in primary attractancy would be recommended for future investigation. This should include the isolation of bacteria, yeasts and fungi associated with *P. radiata* bark and phloem and the subsequent assay of their attractiveness to *H. ater* in the laboratory and/or field. Also investigation of the potential role of the beetle associated fungal organism *L. lundbergii* in conditioning the host for brood development and nutrition should be undertaken.

Identification and investigation of the unknown compound isolated from *H. ater* frass would also be recommended in further understanding the chemical communication aspects of *H. ater*.

Extensive investigation is also required to determine an economic threshold for monitoring purposes. Evaluation of the background population in terms of *H. ater* numbers, and the level of attack, is required to enable forest managers to determine when and what management techniques are to be applied.

The impact of the chopper rolling slash retention technique on *H. ater*, needs to be evaluated under Tasmanian field conditions. This technique is the most cost effective method when combined with strip cultivation. The influence of maintaining the soil-bark interface by chopper rolling requires further investigation to ensure that this practice does not increase *H. ater* breeding sites and activity. Also, an examination of the optimum timing and condition of slash material may be of use in evaluating the effectiveness of this treatment.

An investigation of the susceptibility of seedlings and cuttings (of similar genetic make-up) should be conducted in order to prove or disprove the theory that cuttings are more sensitive to beetle attack.

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Appendix 1

Assessment of Trapping Methods

Traps	Trial 2.1	Trial 2.2	Trial 2.3	Total H.ater/Trial/Trap	Mean
North Pane Trap	34	13	12	59	19.7
East Pane Trap	45	24	5	74	24.7
Large Mesh Cage	4	0	N/A	4	2.0
Small Mesh Cage	4	0	N/A	4	2.0
Large Mesh Billet	5	0	N/A	5	2.5
Small Mesh Billet	3	0	N/A	3	1.5
Cylinder tin trap	N/A	4	0	4	2.0
Cylinder tin trap	N/A	2	0	2	1.0
Cylinder tin trap with base	N/A	2	1	3	1.5
Total H.ater/Trial	95	45	18	**158**	**52.7**

Total number of *H. ater* adult beetles caught on the various traps (Trials 2.1, 2.2 and 2.3)

Billets	Trial 2.1	Trial 2.2	Trial 2.3	Total H.ater/Trial/Trap	Mean
North Pane Trap	115	77	77	269	89.6
East Pane Trap	585	137	40	762	254.0
Large Mesh Cage	299	15	N/A	314	157.0
Small Mesh Cage	38	82	N/A	120	60.0
Large Mesh Billet	348	67	N/A	415	207.5
Small Mesh Billet	225	32	N/A	257	128.5
Control	590	35	47	672	224.0
Control	171	45	26	242	80.7
Cylinder tin trap	N/A	8	36	44	22.0
Cylinder tin trap	N/A	2	16	18	9.0
Cylinder tin trap with base	N/A	N/A	N/A	0	0
Total H.ater/Trial	2371	500	242	**3113**	**1037.7**

Total number of *H. ater* adult beetles caught on the billets corresponding to the various traps (Trials 2.1, 2.2 and 2.3)

Appendix 2

Arrival of *H. ater* adult beetles to *P. radiata* material

Billet Number	Number of Beetles	Male	Female
1	27	13	14
2	27	8	19
3	7	3	4
4	10	4	6
5	13	4	9
6	11	4	7
7	55	32	23
8	28	18	10
9	10	2	8
10	12	6	6
11	35	19	16
12	69	32	37
TOTAL	304	145	159
	%	48	52

Arrival of *H. ater* adult beetles to *P. radiata* material Trial 3.1

Billet Number	Number of Beetles	Male	Female
481	6	1	5
482	1	0	1
480	2	1	1
488	11	8	3
486	1	1	0
485	3	0	3
479	17	11	6
483	14	9	5
TOTAL	55	31	24
	%	56	44

Arrival of *H. ater* adult beetles to *P. radiata* material Trial 3.2

Billet Number	Number of individual beetles			Number of beetle pairs		
	Total	Male	Female	Total	Male	Female
1	3	2	1	2	2	2
3	13	7	6	0	0	0
4	2	0	2	0	0	0
5	6	3	3	1	1	1
6	3	1	2	0	0	0
TOTAL	27	13	14	3	3	3
	%	48	52	%	50	50

Arrival of *H. ater* adult beetles to *P. radiata* material Trial 3.3

Billet Number	Number of individual beetles			Number of beetle pairs			Number of beetles in a mass		
	Total	Male	Female	Total	Male	Female	Total	Male	Female
1	-	-	-	1	1	1	3	1	2
1	-	-	-	-	-	-	3	2	1
2	2	0	2	0	-	-	-	-	-
3	3	2	1	1	1	1	4	3	1
4	1	1	0	1	2	0	-	-	-
4	-	-	-	1	1	1	-	-	-
5	-	-	-	-	-	-	3	2	1
6	-	-	-	2	2	2	32	18	14
7	3	2	1	-	-	-	8	6	2
7	3	1	2	-	-	-	-	-	-
8	-	-	-	-	-	-	10	8	2
9	1	0	1	-	-	-	-	-	-
10	1	0	1	1	2	0	-	-	-
11	-	-	-	1	2	0	-	-	-
12	3	2	1	-	-	-	-	-	-
TOTAL	17	8	9	8	11	5	63	40	23
	%	47	53	%	69	31	%	63	37

Arrival of *H. ater* adult beetles to *P. radiata* material Trial 3.4

Appendix 3

H. ater adult beetle weights

Month	Sex	n	Mean	SE
April	F	30	0.005	0.00027
April	M	20	0.004	0.00005
December	F	29	0.008	0.00290
December	M	13	0.008	0.00663
January	F	118	0.008	0.01111
January	M	67	0.005	0.02176
May	F	120	0.006	0.00058
May	M	80	0.004	0.00109
November	F	5	0.019	0.00998
November	M	5	0.016	0.01395

Variable	Source	DF	Sum of Square	Mean Square	F Value	P Value
Weight	Date	4	0.00172916	0.00043229	12.27	0.0001
	Sex	1	0.00014812	0.00014812	4.20	0.0409
	Date*Sex	4	0.00013127	0.00003282	0.93	0.4453

Appendix 4

Head capsule widths of *H. ater* four larval instars

Instar I

Number Larvae	Head Capsule Width (mm)
1	0.328
1	0.33825
10	0.35875
9	0.369
7	0.37925
20	0.3895
10	0.39975
5	0.41
1	0.42025
1	0.4305
65	0.383 ± 0.019

Instar II

Number Larvae	Head Capsule Width (mm)
1	0.451
8	0.46125
5	0.4715
6	0.48175
10	0.492
14	0.50225
28	0.5125
5	0.52275
13	0.533
6	0.54325
1	0.5535
4	0.56375
3	0.574
3	0.58425
1	0.5945
108	0.512 ± 0.031

Instar III

Number Larvae	Head Capsule Width (mm)
6	0.615
3	0.6355
2	0.64575
1	0.656
11	0.66625
4	0.6765
1	0.68675
1	0.697
14	0.7175
1	0.72775
2	0.738
2	0.7585
5	0.76875
3	0.779
56	0.695 ± 0.049

Instar IV

Number Larvae	Head Capsule Width (mm)
2	0.78925
3	0.80975
10	0.82
2	0.83025
2	0.8405
3	0.85075
4	0.861
14	0.87125
7	0.8815
5	0.89175
7	0.902
2	0.91225
29	0.9225
1	0.93275
7	0.943
2	0.95325
3	0.9635
14	0.97375
1	0.984
3	1.0045
2	1.025
1	1.09675
1	1.107
2	1.1275
127	0.911 ± 0.064

Appendix 5

Bark Thickness Trial 4.1

Variable	Source	DF	Sum of Square	Mean Square	F Value	P Value
Number of beetles	Model	1	15887.81	15887.81	37.40	0.0001
	Error	12	5097.11	424.76		
	Total	13	20984.93			

Root MSE 20.61 R-square = 0.7571

Dep Mean 198.07 Adj R-square = 0.7369

C.V. 10.41

Variable	DF	Parameter Estimate	SE	T for HO Parameter = 0	P Value
Intercept	1	117.59	14.26	8.24	0.0001
Bark Thickness	1	11.57	1.89	6.11	0.0001

Appendix 6

Soaking Experiment Trial 4.8

Average number of *H. ater* entry holes made in fully submerged water soaked billets per cm^2 and cm^3 of *P. radiata* bark (Trial 4.8)

Treatment	n	Average number entry holes/cm^2	Average number entry holes/cm^3
'Dry' control billet	6	0.00009	0.00003
'Fresh' control billet	6	0.00067	0.00013
'Dry' billet soaked 4 weeks	6	0.00048	0.00017
'Dry' billet soaked 2 weeks	6	0.00060	0.00022
'Dry' billet soaked 1 week	12	0.00037	0.00013
'Fresh' billet soaked 4 weeks	6	0.00026	0.00005
'Fresh' billet soaked 2 weeks	6	0.00049	0.00010
'Fresh' billet soaked 1 week	12	0.00084	0.00021
'Fresh' billet endcoated 4 weeks	6	0.00051	0.00016
'Fresh' billet endcoated 2 weeks	6	0.00072	0.00018
'Fresh' billet endcoated 1 week	12	0.00169	0.00056
	LSD (0.05)	0.00110	0.00030

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	5	0.00000782	0.00000156	1.51	0.1998
	Treatment	10	0.00001751	0.00000175	1.68	0.1023
	Total	15	0.00002533			
Entry Holes per cm^3	Replicate	5	0.00000077	0.00000015	1.45	0.2187
	Treatment	10	0.00000200	0.00000020	1.89	0.0621
	Total	15	0.00000276			

Moisture content of bark and wood of soaked billets before and after treatment and after establishment (Trial 4.8)

Treatment	Initial Bark Moisture Content	Bark Moisture Content After Treatment	Bark Moisture Content After 78 days Establishment	Initial Wood Moisture Content	Wood Moisture Content After Treatment	Wood Moisture Content After 78days Establishment
Dry control billet	39.89	N/A	31.49	33.79	N/A	29.68
Fresh control billet	50.71	N/A	43.78	65.68	N/A	55.13
Dry billet soaked 4 weeks	63	73.91	48.81	57.21	60.75	48.41
Dry billet soaked 2 weeks	44.62	64.13	48.5	27.7	43.85	45.45
Dry billet soaked 1 week	26.77	48.78	41.24	26.38	31.01	44.58
Fresh billet soaked 4 weeks	37.85	57.58	38.99	57.32	58.54	49.02
Fresh billet soaked 2 weeks	47.64	61.43	49.38	62.57	67.23	48.35
Fresh billet soaked 1 week	33.04	49.21	32.23	52.87	58.12	49.02
Fresh billet endcoated 4 weeks	57.53	67.05	62.8	61.3	60.9	54
Fresh billet endcoated 2 weeks	58.83	63.94	59.66	65.02	59.15	58.82
Fresh billet endcoated 1 week	58.7	60.34	62.21	57	52.46	57.72
Extra Dry billet soaked 1 week	43.48	64.02	36.36	25.53	37.27	31.57
Extra Fresh billet soaked 1 week	36.21	53.06	40.57	62.72	62.84	50.47
Extra Fresh billet endcoated 1 week	56.06	57.82	54.33	59.99	60.28	50.77

Appendix 7

Soaking Experiment Trial 4.9

Average number of *H. ater* entry holes made in fully submerged water soaked billets per cm^2 and cm^3 of *P. radiata* bark (Trial 4.9)

Treatment	n	Average number entry holes/cm^2	Average number entry holes/cm^3
'Dry' control billet	6	0.0007	0.0003
'Fresh' control billet	6	0.0017	0.0006
'Dry' billet soaked 8 weeks	6	0.0026	0.0009
'Dry' billet soaked 1 week	6	0.0011	0.0004
'Fresh' billet soaked 8 weeks	6	0.0024	0.0006
'Fresh' billet soaked 1 week	6	0.0018	0.0005
'Fresh' billet endcoated 8 weeks	6	0.0015	0.0006
'Fresh' billet endcoated 1 week	6	0.0031	0.0013
	LSD (0.05)	0.0019	0.0007

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	5	0.00001602	0.00000320	1.27	0.2981
	Treatment	7	0.00002726	0.00000389	1.55	0.1847
	Total	12	0.00004328			
Entry Holes per cm^3	Replicate	5	0.00000177	0.00000035	0.94	0.4652
	Treatment	7	0.00000433	0.00000062	1.65	0.1527
	Total	12	0.00000610			

Moisture content of bark and wood of soaked billets before and after treatment and after establishment (Trial 4.9)

Treatment	Initial Bark Moisture Content	Bark Moisture Content After Treatment	Bark Moisture Content After 50days Establishment	Initial Wood Moisture Content	Wood Moisture Content After Treatment	Wood Moisture Content After 50days Establishment
Dry control billet	45.02	N/A	40.32	28.63	N/A	30.18
Fresh control billet	49.24	N/A	40.63	54.3	N/A	45.64
Dry billet soaked 8 weeks	59.65	70.6	50.7	57.24	59.13	45.47
Dry billet soaked 1 week	54.84	64.75	43.73	32.14	41.86	30.62
Fresh billet soaked 8 weeks	57.05	78.85	53.3	60.34	61.92	55.23
Fresh billet soaked 1 week	52.63	66.66	53.45	59.6	62.83	57.23
Fresh billet endcoated 8 weeks	60.04	65.93	58.77	63.81	50.78	49.72
Fresh billet endcoated 1 week	58.52	58.78	59.85	62.88	57.3	46.48

Appendix 8

Average number of *H. ater* entry holes made and adult beetles present in fully submerged water soaked billets per cm^2 and cm^3 of *P. radiata* bark (Trial 4.10)

Treatment	n	Average number entry holes/cm^2	Average number entry holes/cm^3	Average number beetles/cm^2	Average number beetles/cm^3
'Dry' control billet	6	0.01129	0.00456	0.05737	0.02321
'Fresh' control billet	6	0.00539	0.00133	0.02238	0.00551
'Dry' billet soaked 6 weeks	6	0.00182	0.0004	0.0194	0.00427
'Dry' billet soaked 4 weeks	6	0.0135	0.00498	0.03086	0.01111
'Dry' billet soaked 2 weeks	6	0.006	0.00254	0.018	0.00758
'Dry' billet soaked 1 week	6	0.01094	0.00354	0.10293	0.03263
'Fresh' billet soaked 6 weeks	6	0.01057	0.00240	0.071	0.01631
'Fresh' billet soaked 4 weeks	6	0.01432	0.00398	0.11773	0.03162
'Fresh' billet soaked 2 weeks	6	0.00773	0.00178	0.05019	0.01141
'Fresh' billet soaked 1 week	6	0.00929	0.00275	0.17346	0.05328
'Fresh' billet endcoated 6 weeks	6	0.00920	0.00231	0.03214	0.00814
'Fresh' billet endcoated 4 weeks	6	0.01160	0.00394	0.06290	0.02146
'Fresh' billet endcoated 2 weeks	6	0.00540	0.00152	0.02362	0.00654
'Fresh' billet endcoated 1 week	6	0.01158	0.00430	0.05486	0.02051
	LSD (0.05)	0.00590	0.00210	0.10040	0.03160

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	5	0.00183611	0.00036722	13.81	0.0001
	Treatment	13	0.00109803	0.00008446	3.18	0.0010
	Total	18	0.00293414			
Entry Holes per cm^3	Replicate	5	0.00021995	0.00004399	12.89	0.0001
	Treatment	13	0.0001568	0.00001206	3.53	0.0003
	Total	18	0.00037675			
Number Beetles per cm^2	Replicate	5	0.06656173	0.01331235	1.76	0.1343
	Treatment	13	0.16692043	0.01284000	1.69	0.0833
	Total	18	0.23348216			
Number Beetles per cm^3	Replicate	5	0.00677725	0.00135545	1.81	0.1239
	Treatment	13	0.01548094	0.00119084	1.59	0.1119
	Total	18	0.02225819			

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	5	0.00183611	0.00036722	11.65	0.0001
	Duration of Soaking	4	0.00049431	0.00012358	3.92	0.0061
	Total	9	0.00233042			
Entry Holes per cm^3	Replicate	5	0.00021995	0.00004399	10.47	0.0001
	Duration of Soaking	4	0.00006769	0.00001692	4.03	0.0052
	Total	9	0.00028764			
Number Beetles per cm^2	Replicate	5	0.06656173	0.01331235	1.77	0.1304
	Duration of Soaking	4	0.10166512	0.02541628	3.37	0.0137
	Total	9	0.16822686			
Number Beetles per cm^3	Replicate	5	0.00677725	0.00135545	1.85	0.1134
	Duration of Soaking	4	0.01005809	0.00251452	3.43	0.0125
	Total	9	0.01683534			

Moisture content of bark and wood of soaked billets before and after treatment (Trial 4.10)

Treatment	Initial Bark Moisture Content	Bark Moisture Content After Treatment	Initial Wood Moisture Content	Wood Moisture Content After Treatment
Dry control billet	31.19	N/A	21.94	N/A
Fresh control billet	34.66	N/A	53.97	N/A
Dry billet soaked 6 weeks	11.29	49.57	10.22	53.04
Dry billet soaked 4 weeks	35.31	57.34	24.12	34.36
Dry billet soaked 2 weeks	54.16	51.97	17.12	32.33
Dry billet soaked 1 week	21.12	25.02	20.83	16.04
Fresh billet soaked 6 weeks	39.69	50.64	54.76	52.92
Fresh billet soaked 4 weeks	45.91	56.07	56.55	55.53
Fresh billet soaked 2 weeks	26.20	42.11	51.98	57.69
Fresh billet soaked 1 week	35.54	42.08	53.51	54.48
Fresh billet endcoated 6 weeks	55.25	56.25	58.45	61.41
Fresh billet endcoated 4 weeks	62.57	62.71	57.99	56.28
Fresh billet endcoated 2 weeks	42.44	43.20	54.44	52.95
Fresh billet endcoated 1 week	53.25	55.37	58.02	54.40

Appendix 9

Assessment of Potential Volatile Compounds Trial 5.1

Average number of entry holes and beetles per cm^2 and cm^3 of *P. radiata* material for each treatment

Treatment	n	Average number entry holes per cm^2	Average number entry holes per cm^3	Average number beetles per cm^2	Average number beetles per cm^3
control	12	0.08	0.007	0.074	0.006
alpha-pinene	4	0.026	0.007	0.018	0.005
cis-verbenol	4	0.035	0.009	0.020	0.005
trans-verbenol	4	0.030	0.007	0.021	0.005
verbenone	4	0.032	0.008	0.019	0.005

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	3	0.00014744	0.00004915	6.15	0.0039
	Treatment	4	0.00001689	0.00000422	0.53	0.7160
	Total	7	0.00016433			
Entry Holes per cm^3	Replicate	3	0.00001614	0.00000538	7.36	0.0016
	Treatment	4	0.00000182	0.00000046	0.62	0.6507
	Total	7	0.00001796			
Number Beetles per cm^2	Replicate	3	0.00015302	0.00005101	2.99	0.0553
	Treatment	4	0.00001108	0.00000277	0.16	0.9549
	Total	7	0.0001641			
Number Beetles per cm^3	Replicate	3	0.00001238	0.00000413	3.69	0.0291
	Treatment	4	0.00000045	0.00000011	0.10	0.9814
	Total	7	0.00001283			
Number Male Beetles per cm^2	Replicate	3	0.00005113	0.00001704	4.20	0.0186
	Treatment	4	0.00000414	0.00000104	0.25	0.9032
	Total	7	0.00005528			
Number Male Beetles per cm^3	Replicate	3	0.00000429	0.00000143	4.98	0.0097
	Treatment	4	0.00000022	0.00000005	0.19	0.9409
	Total	7	0.00000451			
Number Female Beetles per cm^2	Replicate	3	0.00002842	0.00000947	1.67	0.2046
	Treatment	4	0.00000467	0.00000117	0.21	0.9320
	Total	7	0.00003308			
Number Female Beetles per cm^3	Replicate	3	0.00000219	0.00000073	2.03	0.1425
	Treatment	4	0.00000018	0.00000005	0.13	0.9711
	Total	7	0.00000238			

Appendix 10

Assessment of Potential Volatile Compounds Field Trial 5.2

Average number of entry holes and beetles per cm^2 and cm^3 of *P. radiata* material for each treatment

Treatment	n	Average number entry holes per cm^2	Average number entry holes per cm^3	Average number beetles per cm^2	Average number beetles per cm^3	Average number male beetles per cm^2	Average number male beetles per cm^3	Average number female beetles per cm^2	Average number female beetles per cm^3
cis-verbenol	5	0.023694	0.008558	0.016079	0.005861	0.009732	0.003562	0.006347	0.002300
verbenone	5	0.023056	0.007750	0.033374	0.011261	0.019580	0.006526	0.013794	0.004735
control	15	0.025052	0.008912	0.024491	0.008055	0.015494	0.005129	0.009174	0.002985
trans-verbenol	5	0.018490	0.006374	0.020086	0.006786	0.011331	0.003906	0.008754	0.002880
α-pinene	5	0.024575	0.009463	0.025032	0.009382	0.014268	0.005540	0.010756	0.003978

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	4	0.00288086	0.00072022	12.99	0.0001
	Treatment	4	0.00016843	0.00004211	0.76	0.5611
	Total	8	0.00304929			
Entry Holes per cm^3	Replicate	4	0.00094326	0.00023581	14.38	0.0001
	Treatment	4	0.00003233	0.00000808	0.49	0.7410
	Total	8	0.00097559			
Number Beetles per cm^2	Replicate	4	0.00147191	0.00036798	2.17	0.1007
	Treatment	4	0.00083857	0.00020964	1.24	0.3202
	Total	8	0.00231049			
Number Beetles per cm^3	Replicate	4	0.00052737	0.00013184	4.99	0.0040
	Treatment	4	0.00009148	0.00002287	0.87	0.4973
	Total	8	0.00061886			
Number Male Beetles per cm^2	Replicate	4	0.00059805	0.00014951	3.02	0.0361
	Treatment	4	0.00030797	0.00007699	1.55	0.2164
	Total	8	0.00090602			
Number Male Beetles per cm^3	Replicate	4	0.00021509	0.00005377	7.16	0.0005
	Treatment	4	0.00002900	0.00000725	0.97	0.4431
	Total	8	0.00024409			
Number Female Beetles per cm^2	Replicate	4	0.00021016	0.00005254	1.23	0.3207
	Treatment	4	0.00015393	0.00003848	0.90	0.4759
	Total	8	0.00036409			
Number Female Beetles per cm^3	Replicate	4	0.00006985	0.00001746	2.73	0.0506
	Treatment	4	0.00001992	0.00000498	0.78	0.5488
	Total	8	0.00008977			

Appendix 11

Assessment of Potential Volatile Compounds Trial 5.3

Average number of entry holes and beetles per cm^2 and cm^3 of *P. radiata* material for each treatment

Treatment	n	Average number entry holes per cm^2	Average number entry holes per cm^3	Average number beetles per cm^2	Average number beetles per cm^3
control	15	0.075745	0.030716	0.072755	0.02962
cis-verbenol	5	0.044820	0.017375	0.084545	0.030925
trans-verbenol	5	0.035368	0.013808	0.041586	0.016279
verbenone	5	0.040445	0.015576	0.076264	0.026622
α-pinene	5	0.027793	0.012718	0.029648	0.012529
sulcatol	3	0.029260	0.010876	0.053410	0.019208
MCH	3	0.019544	0.007546	0.027247	0.010434
MCOL	3	0.204429	0.088312	0.045851	0.023267

Variable	Source	DF	Sum of square	Mean square	F Value	P Value	LSD (0.05)
Entry Holes per cm^2	Replicate	4	0.08281969	0.02070492	3.55	0.0167	0.1063
	Treatment	7	0.08111764	0.01158823	1.98	0.0884	
	Total	11	0.16393732				
Entry Holes per cm^3	Replicate	4	0.0103462	0.00258655	2.74	0.0454	0.0427
	Treatment	7	0.01542405	0.00220344	2.34	0.0479	
	Total	11	0.02577024				
Number Beetles per cm^2	Replicate	4	0.07162748	0.01790687	5.35	0.0021	0.0805
	Treatment	7	0.01964988	0.00280713	0.84	0.5639	
	Total	11	0.09127736				
Number Beetles per cm^3	Replicate	4	0.00781445	0.00195361	3.89	0.011	0.0312
	Treatment	7	0.00258077	0.00036868	0.73	0.6452	
	Total	11	0.01039522				

Appendix 12

Assessment of potential activity of volatile compounds (Field Trial 5.2)

Average number of *H. ater* entry holes per *P. radiata* billet over time for each treatment (Figure 5.8)

Treatment	5 days	10 days	14 days	19 days	23 days
control	0.4	10.9	4.9	6.4	4.7
α-pinene	0.6	10.6	2.6	5.4	6.8
cis-verbenol	0.2	10.6	7.4	6.8	0.2
trans-verbenol	0.4	11.2	4.6	2.0	2.8
verbenone	0.2	12.2	6.2	5.2	2.6

Appendix 13

Assessment of potential activity of volatile compounds (Field Trial 5.3)

Average number of *H. ater* entry holes per *P. radiata* billet over time for each treatment (Figure 5.9)

Treatment	5 days	11 days	15 days	20 days	25 days	30 days	42 days
control	8.73	11	11.4	14.9	0.9	3.8	1.13
α-pinene	3.8	6.4	1.4	4.0	4.4	1.4	2.0
cis-verbenol	7.4	12.4	1.6	3.2	4.6	0.8	0.8
trans-verbenol	6.4	7.0	8.4	9.4	1.8	0	1.6
verbenone	6.0	15	9.2	6.2	0.8	1.2	2.4
sulcatol	6.0	10.3	9.7	1.7	1.7	0	0.67
MCH	4.3	6.0	4.7	3.7	0	0.3	0.67
MCOL	15.3	22.7	30.3	1.7	17	0	0

Appendix 14

Assessment of Potential Volatile Compounds Field Trial 5.5

Average number of entry holes and beetles per cm^2 and cm^3 of *P. radiata* material for each treatment

Treatment	n	Average number entry holes per cm^2	Average number entry holes per cm^3	Average number beetles per cm^2	Average number beetles per cm^3	Average number male beetles per cm^2	Average number male beetles per cm^3	Average number female beetles per cm^2	Average number female beetles per cm^3
control	6	0.00846	0.00333	0.02300	0.00896	0.01402	0.00541	0.00897	0.00354
cis-verbenol	2	0.01198	0.00496	0.02318	0.00906	0.01237	0.00484	0.01080	0.00422
verbenone	2	0.00794	0.00317	0.03108	0.01234	0.01896	0.00753	0.01211	0.00481
trans-verbenol	2	0.01030	0.00400	0.03141	0.01220	0.02008	0.00780	0.01133	0.00440
α-pinene	2	0.01230	0.00543	0.02442	0.01071	0.01396	0.00612	0.00987	0.00432
sulcatol	2	0.01548	0.00655	0.03141	0.01388	0.01626	0.00718	0.01514	0.00670
MCH	2	0.00980	0.00412	0.01000	0.00406	0.00639	0.00258	0.00367	0.00147

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	1	0.00000059	0.00000059	0.02	0.881
	Treatment	6	0.00009899	0.00001650	0.66	0.683
	Total	7	0.00009957			
Entry Holes per cm^3	Replicate	1	0.00000023	0.00000023	0.05	0.829
	Treatment	6	0.00002164	0.00000361	0.76	0.615
	Total	7	0.00002188			
Number Beetles per cm^2	Replicate	1	0.00057563	0.00057563	6.64	0.028
	Treatment	6	0.00071137	0.00011856	1.37	0.315
	Total	7	0.00128700			
Number Beetles per cm^3	Replicate	1	0.00008252	0.00008252	5.16	0.046
	Treatment	6	0.00013058	0.00002176	1.36	0.317
	Total	7	0.00021310			
Number Male Beetles per cm^2	Replicate	1	0.00024859	0.00024859	8.81	0.014
	Treatment	6	0.00025097	0.00004183	1.48	0.277
	Total	7	0.00049956			
Number Male Beetles per cm^3	Replicate	1	0.00003513	0.00003513	7.61	0.020
	Treatment	6	0.00004138	0.00000690	1.49	0.274
	Total	7	0.00007651			
Number Female Beetles per cm^2	Replicate	1	0.00006326	0.00006326	2.89	0.1201
	Treatment	6	0.00015299	0.0000255	1.16	0.396
	Total	7	0.00021626			
Number Female Beetles per cm^3	Replicate	1	0.00000924	0.00000924	2.11	0.177
	Treatment	6	0.00003056	0.00000509	1.16	0.396
	Total	7	0.00003980			

Appendix 15

Assessment of Volatile Compounds Laboratory Trials

Compound	Sex	Rep	Positive	Negative	No response
α-pinene	M	10	2	4	4
α-pinene	F	10	2	2	6
exo-brevicomin	M	10	5	2	3
exo-brevicomin	F	10	4	1	5
frontalin	M	10	3	2	5
frontalin	F	10	4	1	5
verbenene	M	10	0	1	9
verbenene	F	10	3	3	4
sulcatol	M	10	2	1	7
sulcatol	F	10	3	1	6
trans-verbenol	M	10	0	4	6
trans-verbenol	F	10	3	4	3
cis-verbenol	M	10	7	0	3
cis-verbenol	F	10	2	1	7
verbenone	M	10	2	1	7
verbenone	F	10	4	1	5
MCOL	M	10	2	0	8
MCOL	F	10	7	0	3
MCH	M	10	2	2	6
MCH	F	10	1	5	4
Pine bark	M	10	10	0	0
Pine bark	F	10	10	0	0
α-pinene & MCOL	M	10	1	2	7
α-pinene & MCOL	F	10	0	1	9
α-pinene & *cis*-verbenol	M	10	2	0	8
α-pinene & *cis*-verbenol	F	10	0	4	6
Frass	M	10	3	2	5
Frass	F	10	2	5	3

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Positive	Compound	13	148.86	11.45	3.68	0.013
	Beetle Sex	1	0.57	0.57	0.18	0.675
	Total	14	149.73			
Negative	Compound	13	43.71	3.36	2.11	0.096
	Beetle Sex	1	2.29	2.29	1.43	0.252
	Total	14	46.00			
No Response	Compound	13	91.43	7.03	1.95	1.207
	Beetle Sex	1	5.14	5.14	1.43	0.254
	Total	14	96.57			

Volatile compounds detected from feeding *H. ater* beetles on *P. radiata* over time (Total ion current)

Days	α-pinene	β-pinene + sabinene	myrcene	δ-3-carene	terpinolene	cymene	fenchone	camphor	isopinocamphone	unknown
0	46833333	1.52E+09	11600000	68223333	7932000	0	0	0	87000	0
2	32766667	1.17E+09	7103333	31876667	4944333	0	0	215666.7	278333.3	0
4	42923333	1.53E+09	9596667	69500000	6510000	533333.3	144666.6	764333.3	651666.7	0
6	26793333	95233333	6312667	47916667	4290000	346666.7	39566.67	742333.3	467333.3	0
10	21286667	81233333	5421667	42510000	3323333	430000	145000	2203333	771333.3	83666.67
15	17900000	66033333	4282333	37800000	2558667	176000	284333.3	3880000	959333.3	1022000
21	11586667	35866667	2765667	38666667	2011667	210000	443000	3016667	1140000	1467667
24	5476667	24333333	1914667	26463333	1624000	156666.7	613000	2241667	1304667	1694000

Volatile compounds detected from *P. radiata* over time (Total ion current)

Days	α-pinene	β-pinene + sabinene	terpinolene	myrcene	δ-3-carene	cymene	camphor	isopinocamphone
0	52800000	16500000	7083333	12986667	71666667	0	0	88333.33
2	29866667	10500000	4503333	7310000	46533333	0	99666.67	206333.3
4	31483333	11400000	5333333	8166667	54366667	0	238666.7	368000
6	20133333	74200000	3646667	5430000	38603333	0	127666.7	203333.3
10	16000000	61266667	2911667	3996667	33166667	0	155333.3	262666.7
15	14216667	53500000	2087000	2450000	27966667	700000	303333.3	541333.3
21	13660000	51733333	1620000	2150000	27490000	483333.3	1110000	974666.7
24	13033333	50500000	1682000	2086667	28800000	546666.7	1896333	1105667

Appendix 17

Bacterial/Yeast Field Trial 6.1

Treatment	Entry Holes		Number beetles	
	cm^2	cm^3	cm^2	cm^3
Control billet	0.018	0.005	0.027	0.010
Billet + Nutrient Agar Control	0.011	0.005	0.009	0.004
Billet + Nutrient Broth Control	0.009	0.004	0.009	0.004
Billet + Phloem Broth Control	0.018	0.008	0.023	0.010
Billet + Nutrient Agar + Isolate 1	0.006	0.002	0.005	0.002
Billet + Nutrient Agar + Isolate 2	0.009	0.004	0.009	0.005
Billet + Nutrient Agar + Isolate 3	0.015	0.006	0.020	0.008
Billet + Nutrient Agar + Isolate 8	0.006	0.002	0.006	0.002
Billet + Nutrient Agar + Isolate 11	0.007	0.003	0.017	0.007
Billet + Nutrient Broth + Isolate 1	0.012	0.004	0.009	0.003
Billet + Nutrient Broth + Isolate 2	0.005	0.002	0.003	0.001
Billet + Nutrient Broth + Isolate 3	0.011	0.005	0.016	0.007
Billet + Nutrient Broth + Isolate 8	0.010	0.004	0.016	0.007
Billet + Nutrient Broth + Isolate 11	0.016	0.006	0.050	0.019
Billet + Phloem Broth +Isolate 1	0.021	0.009	0.040	0.018
Billet + Phloem Broth +Isolate 2	0.006	0.002	0.008	0.005
Billet + Phloem Broth +Isolate 3	0.010	0.003	0.018	0.006
Billet + Phloem Broth +Isolate 8	0.017	0.009	0.021	0.011
Billet + Phloem Broth +Isolate 11	0.010	0.005	0.027	0.015
Mean $\pm$ SE	0.011 ± 0.005	0.005 ± 0.002	0.018 ± 0.012	0.008 ± 0.005

Appendix 18

Bacterial/Yeast Field Trial 6.3

Treatment	Entry Holes	
	cm^2	cm^3
Control billet	0.037	0.013
Billet + Nutrient Agar Control	0.031	0.010
Billet + Nutrient Broth Control	0.030	0.013
Billet + Phloem Broth Control	0.022	0.007
Billet + Nutrient Agar + Isolate 1	0.023	0.008
Billet + Nutrient Agar + Isolate 5	0.028	0.014
Billet + Nutrient Agar + Isolate 6	0.028	0.012
Billet + Nutrient Agar + Isolate 7	0.039	0.017
Billet + Nutrient Agar + Isolate 9	0.030	0.016
Billet + Nutrient Broth + Isolate 1	0.027	0.015
Billet + Nutrient Broth + Isolate 5	0.032	0.016
Billet + Nutrient Broth + Isolate 6	0.025	0.015
Billet + Nutrient Broth + Isolate 7	0.043	0.021
Billet + Nutrient Broth + Isolate 9	0.032	0.019
Billet + Phloem Broth +Isolate 1	0.034	0.016
Billet + Phloem Broth +Isolate 5	0.042	0.025
Billet + Phloem Broth +Isolate 6	0.048	0.026
Billet + Phloem Broth +Isolate 7	0.030	0.017
Billet + Phloem Broth +Isolate 9	0.023	0.009
Mean $\pm$ SE	0.032 ± 0.007	0.015 ± 0.005

Appendix 19

Bacterial/Yeast Inoculated billets

Treatment	Number Entry Holes		Number beetles	
	cm^2	cm^3	cm^2	cm^3
Billet inoculated with Isolate 1	0.011	0.004	0.018	0.007
Billet inoculated with Isolate 1	0.010	0.004	0.017	0.007
Billet inoculated with Isolate 1	0.006	0.002	0.012	0.005
Billet inoculated with Isolate 1	0.007	0.003	0.011	0.005
Billet inoculated with Isolate 1	0.012	0.005	0.019	0.008
Billet inoculated with Nutrient Broth control	0.007	0.003	0.011	0.005
Uninoculated control billet	0.009	0.004	0.010	0.004

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	4	0.00025375	0.00006344	1.86	0.1505
	Treatment	6	0.00014135	0.00002356	0.69	0.6595
	Total	10	0.00039509			
Entry Holes per cm^3	Replicate	4	0.00003416	0.00000854	1.39	0.2662
	Treatment	6	0.00002519	0.0000042	0.68	0.6635
	Total	10	0.00005935			
Number Beetles per cm^2	Replicate	4	0.00193729	0.00048432	4.27	0.0095
	Treatment	6	0.00044141	0.00007357	0.65	0.6909
	Total	10	0.00237870			
Number Beetles per cm^3	Replicate	4	0.00030539	0.00007635	4.03	0.0122
	Treatment	6	0.00006332	0.00001055	0.56	0.7600
	Total	10	0.00036870			

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	4	0.00025375	0.00006344	1.92	0.1337
	Inoculation	1	0.00000245	0.00000245	0.07	0.7873
	Total	5	0.00025619			
Entry Holes per cm^3	Replicate	4	0.00003416	0.00000854	1.45	0.2437
	Inoculation	1	0.00000118	0.00000118	0.20	0.6575
	Total	5	0.00003534			
Number Beetles per cm^2	Replicate	4	0.00193729	0.00048432	4.59	0.0054
	Inoculation	1	0.00010685	0.00010685	1.01	0.3224
	Total	5	0.00204414			
Number Beetles per cm^3	Replicate	4	0.00030539	0.00007635	4.39	0.0067
	Inoculation	1	0.00001384	0.00001384	0.80	0.3797
	Total	5	0.00031922			

Appendix 20

Sterilisation trial 1

Treatment	n	Average Number Entry Holes Per cm^2	Average Number Entry Holes Per cm^3	Average Number Beetles Per cm^2	Average Number Beetles Per cm^3
Autoclave	6	0.0032	0.0064	0.0025	0.0050
S/Sterilise	6	0.0048	0.0096	0.0051	0.0104
Control	6	0.0026	0.0052	0.0025	0.0052

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	1	0.00005711	0.00005711	2.57	0.1309
	Treatment	2	0.00001543	0.00000771	0.35	0.7122
	Total	3	0.00007253			
Entry Holes per cm^3	Replicate	1	0.00022844	0.00022844	2.57	0.1309
	Treatment	2	0.00006169	0.00003085	0.35	0.7122
	Total	3	0.00029013			
Number Beetles per cm^2	Replicate	1	0.00004861	0.00004861	1.16	0.3002
	Treatment	2	0.00002739	0.0000137	0.33	0.7271
	Total	3	0.000076			
Number Beetles per cm^3	Replicate	1	0.00019445	0.00019445	1.16	0.3002
	Treatment	2	0.00010958	0.00005479	0.33	0.7271
	Total	3	0.00030402			

Variable	Source	DF	Sum of square	Mean square	F Value	P Value	LSD(0.05)
Entry Holes per cm^2	Replicate	2	0.00007302	0.00003651	2.88	0.1031	
	Billet Type	2	0.00018491	0.00009246	7.28	0.0112	0.0046
	Sand or Soil	1	0.00002777	0.00002777	2.19	0.1699	
	Billet*Sand or Soil	2	0.00004399	0.00002199	1.73	0.2259	
	Total	7	0.00032969				
Entry Holes per cm^3	Replicate	2	0.00000675	0.00000337	2.34	0.1469	
	Billet Type	2	0.00001775	0.00000887	6.15	0.0181	0.0015
	Sand or Soil	1	0.0000053	0.0000053	3.67	0.0844	
	Billet*Sand or Soil	2	0.000004	0.000002	1.38	0.2946	
	Total	7	0.00003379				
Number Beetles per cm^2	Replicate	2	0.00009427	0.00004713	1.83	0.2101	
	Billet Type	2	0.00024784	0.00012392	4.81	0.0343	0.0065
	Sand or Soil	1	0.00000945	0.00000945	0.37	0.5581	
	Billet*Sand or Soil	2	0.00002658	0.00001329	0.52	0.6118	
	Total	7	0.00037813				
Number Beetles per cm^3	Replicate	2	0.00000936	0.00000468	1.68	0.2353	
	Billet Type	2	0.0000246	0.0000123	4.41	0.0424	0.0021
	Sand or Soil	1	0.00000243	0.00000243	0.87	0.373	
	Billet*Sand or Soil	2	0.0000026	0.0000013	0.47	0.6405	
	Total	7	0.00003899				

Appendix 21

Sterilisation trial 2

Treatment	n	Average number Entry Holes/cm^2	Average number Entry Holes/cm^3	Average number Adult Beetles/cm^2	Average number Adult Beetles/cm^3
Autoclaved billet in sterile sand	3	0.0005	0.0002	0	0
Frozen billet in sterile sand	3	0.0106	0.0033	0.0099	0.0030
Control billet in sterile sand	3	0.0004	0.0001	0.0004	0.0001
Autoclaved billet in soil	3	0.0049	0.0018	0.0010	0.0004
Frozen billet in soil	3	0.0086	0.0030	0.0086	0.0030
Control billet in soil	3	0.0054	0.0020	0.0050	0.0019
Control billet in soil (no bag)	3	0.0196	0.0100	0.0184	0.0095

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	2	0.00004471	0.00002236	1.18	0.3399
	Treatment	6	0.0008011	0.00013352	7.06	0.0021
	Total	8	0.00084582			
Entry Holes per cm^3	Replicate	2	0.0000041	0.00000205	0.71	0.5134
	Treatment	6	0.00020347	0.00003391	11.65	0.0002
	Total	8	0.00020758			
Number Beetles per cm^2	Replicate	2	0.00012239	0.00006119	2.29	0.1441
	Treatment	6	0.00080814	0.00013469	5.03	0.0085
	Total	8	0.00093053			
Number Beetles per cm^3	Replicate	2	0.00001629	0.00000815	1.94	0.1862
	Treatment	6	0.00019846	0.00003308	7.88	0.0013
	Total	8	0.00021476			

Variable	Source	DF	Sum of square	Mean square	F Value	P Value	LSD(0.05)
Entry Holes per cm^2	Replicate	2	0.00007302	0.00003651	2.88	0.1031	
	Billet Type	2	0.00018491	0.00009246	7.28	0.0112	0.0046
	Sand or Soil	1	0.00002777	0.00002777	2.19	0.1699	
	Billet*Sand or Soil	2	0.00004399	0.00002199	1.73	0.2259	
	Total	7	0.00032969				
Entry Holes per cm^3	Replicate	2	0.00000675	0.00000337	2.34	0.1469	
	Billet Type	2	0.00001775	0.00000887	6.15	0.0181	0.0015
	Sand or Soil	1	0.00000530	0.00000530	3.67	0.0844	
	Billet*Sand or Soil	2	0.00000400	0.00000200	1.38	0.2946	
	Total	7	0.00003379				
Number Beetles per cm^2	Replicate	2	0.00009427	0.00004713	1.83	0.2101	
	Billet Type	2	0.00024784	0.00012392	4.81	0.0343	0.0065
	Sand or Soil	1	0.00000945	0.00000945	0.37	0.5581	
	Billet*Sand or Soil	2	0.00002658	0.00001329	0.52	0.6118	
	Total	7	0.00037813				
Number Beetles per cm^3	Replicate	2	0.00000936	0.00000468	1.68	0.2353	
	Billet Type	2	0.00002460	0.00001230	4.41	0.0424	0.0021
	Sand or Soil	1	0.00000243	0.00000243	0.87	0.3730	
	Billet*Sand or Soil	2	0.00000260	0.00000130	0.47	0.6405	
	Total	7	0.00003899				

Appendix 22

Attraction to damaged frozen *P. radiata*

Treatment	Average number entry holes/cm^2	Average number entry holes/cm^3	Average number beetles/cm^2	Average number beetles/cm^3
Frozen billets	0.0064	0.0125	0.0883	0.0382
Fresh billets	0.0036	0.0009	0.0095	0.0022
LSD (0.05)	0.0025	0.0013	0.0097	0.0044

Variable	Source	DF	Sum of square	Mean square	F Value	P Value
Entry Holes per cm^2	Replicate	1	0.00004033	0.00004033	11.43	0.0081
	Treatment	1	0.00002296	0.00002296	6.51	0.0311
	Total	2	0.00006330			
Entry Holes per cm^3	Replicate	1	0.00000574	0.00000574	5.73	0.0403
	Treatment	1	0.00001027	0.00001027	10.24	0.0108
	Total	2	0.00001601			
Number Beetles per cm^2	Replicate	1	0.00093987	0.00093987	16.97	0.0026
	Treatment	1	0.00014008	0.00014008	2.53	0.1463
	Total	2	0.00107995			
Number Beetles per cm^3	Replicate	1	0.00012789	0.00012789	11.17	0.0086
	Treatment	1	0.00006748	0.00006748	5.89	0.0381
	Total	2	0.00019537			

Isolation results of the *Neoparasitylenchus* spp. from adult *H. ater* beetles

Date	Number Beetles	Number of beetles with gravid females	Total number gravid females	Infection gravid females	Average number gravid females/beetle	Number of beetles with 1st stage larvae	Infection 1st stage larvae
7/6/94	21	21	97	1	4.62	0	0
23/6/94	10	10	35	1	3.5	0	0
23/6/94	10	10	34	1	3.4	0	0
23/6/94	10	8	22	0.8	2.75	0	0
23/6/94	10	8	26	0.8	3.25	0	0
19/7/94	10	4	17	0.4	4.25	0	0
12/8/94	35	19	58	0.54	3.05	0	0
15/8/94	14	3	12	0.21	4	0	0
23/8/94	13	8	52	0.62	6.5	1	0.08
28/10/94	40	23	65	0.58	2.83	26	0.65
8/11/94	60	56	268	0.93	4.79	56	0.93
8/11/94	40	34	170	0.85	5	33	0.83
1/12/94	17	9	32	0.53	3.56	13	0.76
12/3/95	3	2	2	0.67	1	0	0
12/3/95	7	5	21	0.71	4.2	1	0.14
16/3/95	17	2	3	0.12	1.5	1	0.06
24/3/95	10	6	17	0.6	2.83	0	0
15/6/95	30	19	75	0.63	3.95	10	0.33
15/6/95	30	21	114	0.7	5.43	2	0.07
15/6/95	30	27	109	0.9	4.04	9	0.3
15/6/95	30	20	86	0.67	4.3	4	0.13
15/6/95	41	18	44	0.44	2.44	10	0.24
Jun94-Jun95	**488**	**333**	**1359**	**0.68**	**4.08**	**166**	**0.34**

Appendix 24

Beetle suppression via trap billets – calculations and assumptions

The following assumptions were involved in the calculations:

Surface area of one seedling (based on dimensions 0.6 cm x 15 cm) = 0.0028 m^2

Surface area of one stump (based on dimensions 25 cm x 60 cm) = 4.7 m^2

Surface area of one billet (based on dimensions 20 cm x 100 cm) = 0.63 m^2

Number of seedlings/hectare = 1250-1430

Number of stumps/hectare = 600-1400

Fifteen billets one metre in length harvested from one ~17 year old *P. radiata* tree

Labour costs of $20/hour, chainsaw use $25/hour, and vehicle use $10/hour

Regime	Slash and stump retention	Removal of slash
Available material (m^2/ha)	7000-11000	3000-7000
Number of billets required	11000-17500	5000-11000
Number of trees required	700-1200	300-700
Establishment labour cost	$29,750-$51,000	$12,750-$29,750
Man hours involved	500-800	200-500

Quantities and costs associated with the use of billet traps as barriers

To leave all the stumps and slash residue following harvesting procedures, the number of billets (dimensions 20 cm x 1 m) required to compete with the available stumps and slash per hectare would be 11000-17500 billets per hectare. Assuming 15 such billets could be harvested from one *P. radiata* tree then 700-1200 trees would be required per hectare. Assuming labour costs for harvest, transport and billet establishment of $42.50 per tree, the labour costs for the procedure would be $29,750-$51,000. This does not include the cost of the trees or the labour costs for the subsequent removal of the billets.