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VITAL VOLATILES -  
HOST LOCATION IN PARASITIC WASPS  
ATTACKING BARK BEETLES



EVA M. PETTERSSON

Chemical Ecology  
Göteborg University  
2000



*Cover photography:*

A parasitic wasp (hymenopteran parasitoid, natural size ca 4 mm) *Rhopalicus tutela* ovipositing on a bark beetle larva concealed under the bark of a Norway spruce. Note the ovipositor (close to the middle leg) penetrating the bark (photography by Göran Birgersson).

Göteborg University  
Faculty of Science  
2000



Dissertation

**VITAL VOLATILES -  
HOST LOCATION IN PARASITIC WASPS  
ATTACKING BARK BEETLES**

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Avhandling för filosofie doktorsexamen i Kemisk Ekologi som enligt beslut av Naturvetenskapliga Fakultetsnämnden kommer att försvaras offentligt fredag den 15 december 2000 kl 10:00 i Föreläsningssalen, Botaniska Institutionen, Carl Skottsbergs Gata 22 B, Göteborg. Fakultetsopponent: Professor Ted C. J. Turlings, University of Neuchâtel, Schweiz.

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**VITAL VOLATILES - HOST LOCATION  
IN PARASITIC WASPS ATTACKING BARK BEETLES**

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**ABSTRACT** – Parasitoids are insects that spend their immature stages feeding on other arthropods and eventually kill their hosts. Parasitic wasps (Hymenopteran parasitoids) of bark beetles (Coleoptera: Scolytidae) have a remarkable ability to locate and oviposit on hosts which are concealed under the bark of coniferous trees. The trees are damaged or killed by bark beetles, which introduce pathogenic microorganisms into the phloem where they breed. Despite the lack of visual cues, the parasitoids distinguish bark beetle attacked trees containing susceptible hosts (mature larvae present in pupal chambers).

The aim of this thesis was to identify the host location cues used by four species of bark beetle parasitoids. Synthetic attractants for these parasitoids were successfully developed. Electron microscopy (SEM and TEM) revealed several types of odour perceptive sensilla on the parasitoid antennae. Volatile chemical samples collected from conifers with, or without, bark beetle larvae were analysed chemically (GC-MS) and electrophysiologically (GC-EAD). Monoterpene hydrocarbons were the predominant volatile compounds of healthy conifers, while the presence of oxygenated monoterpenes indicated bark beetle infested trees. A limited number of compounds, primarily oxygenated monoterpenes, elicited detectable neural (EAD) activity in the parasitoid antennae. EAD-active compounds were used to prepare synthetic baits which attracted parasitoid females in behavioural tests, including both Y-olfactometer and wind tunnel bioassays. The attractive compounds seemed not to arise from the parasitoid hosts, the bark beetle larvae, but from the host-plant complex, involving microorganisms.

In addition to having ecological and evolutionary interest, increased knowledge of the host location mechanisms in these parasitoids is potentially useful in developing environmentally safe control methods against epidemic outbreaks of bark beetles breeding in conifers.

**KEYWORDS:** Parasitoids, Hymenoptera, Pteromalidae, *Rhopalicus tutela*, *Roptrocerus mirus*, *Roptrocerus xylophagorum*, Braconidae, *Coeloides bostrichorum*, host location, odours, oxygenated monoterpenes, synthetic attractants, antennal morphology, chemical analysis, electrophysiology, bioassay, bark beetles, Scolytidae, conifers

♪ Till Claes ♪





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## List of papers

This thesis is based on the following papers, referred to by their Roman numbers:

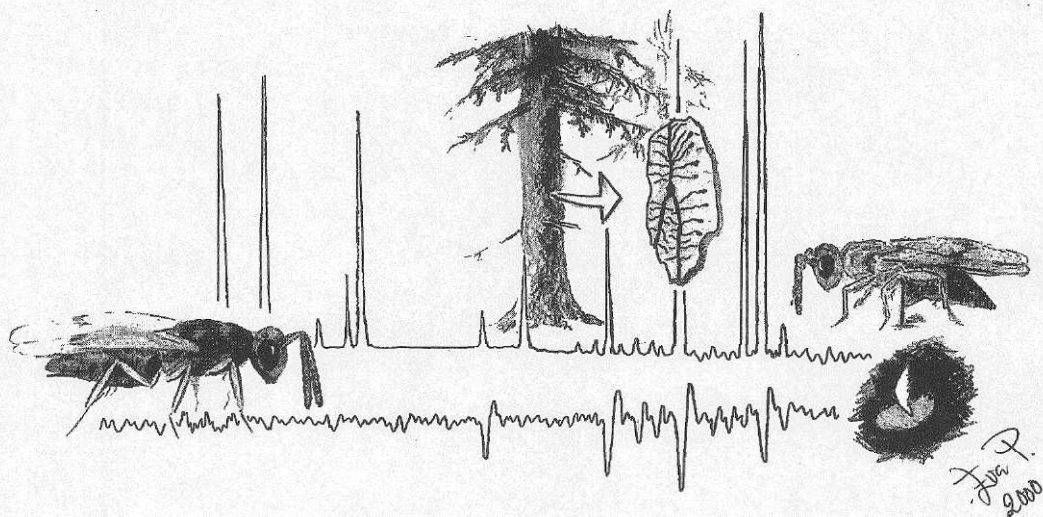
- I PETERSSON E.M., HALLBERG E., and BIRGERSSON G. (2001) Evidence for the importance of odor-perception in the parasitoid *Rhopalicus tutela* (Walker) (Hymenoptera: Pteromalidae). *Journal of Applied Entomology*, accepted for publication
- II PETERSSON E.M. Volatile attractants for three Pteromalid parasitic wasps attacking concealed spruce bark beetles. Manuscript submitted to *Chemoecology*
- III PETERSSON E.M., SULLIVAN B.T., ANDERSON P., BERISFORD C.W., and BIRGERSSON G. (2000) Odor perception in bark beetle parasitoid *Roptrocerus xylophagorum* (Ratzeburg) (Hymenoptera: Pteromalidae) exposed to host associated volatiles. *Journal of Chemical Ecology* 26 (11): in press
- IV SULLIVAN B.T., PETERSSON E.M., SELTMANN K.C., AND BERISFORD C.W. (2000) Attraction of the bark beetle parasitoid *Roptrocerus xylophagorum* (Hymenoptera: Pteromalidae) to host-associated olfactory cues. *Environmental Entomology* 29 (6): in press
- V PETERSSON E.M., BIRGERSSON G., and WITZGALL P. Synthetic attractants for the bark beetle parasitoid *Coeloides bostrichorum* Giraud (Hymenoptera: Braconidae). Manuscript submitted to *Naturwissenschaften*
- VI PETERSSON E.M. Volatiles collected in short-range from potential hosts of *Rhopalicus tutela* (Hym.: Pteromalidae), a bark beetle parasitoid. Manuscript

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Papers I and III are based on research ideas by all authors. I compiled Paper I, and am responsible for the experimental design, field and laboratory work (except the electronmicroscopy), and statistical analysis. In Papers III, I am responsible for the compilation of the manuscript, the electrophysiology and associated chemical analyses. I took part in the planning and the compilation of Paper IV. I am responsible for the experimental design, field and laboratory work, data analysis, and the compilation of Paper V. I am solely responsible for Papers II and VI.

A doctoral thesis at a University in Sweden is produced either as a monograph or as a collection of papers. At the Faculty of Science it is usually done in the latter manner, where the introductory part constitutes the formal thesis which summarises the attached papers. These have already been published or are manuscripts in press, submitted or in preparation.

## Objectives



A female parasitic wasp (hymenopteran parasitoid) is attracted to a bark beetle infested spruce tree from a distance and accurately locates a host under the bark, despite the lack of obvious visual cues on the bark surface. The wasp then penetrates the bark with her ovipositor. An egg is laid on, or near, the bark beetle larva, which is killed during the development of the parasitoid larva. GC-EAD analysis shows that only a limited number of volatile compounds, in the complex odour bouquet surrounding the bark beetle larvae, elicit neural activity in the parasitoid antennae (illustration inspired by Eva Meiners).

This illustration gives a brief introduction to the life-history of parasitic wasps attacking bark beetle larvae concealed under the bark of conifers. The aim of this thesis was to determine if host location by larval parasitoids of bark beetles is mediated by volatiles, and then to determine the source and identity of semiochemicals required for host location.

## - INTRODUCTION -

### Parasitoids

#### What is a Parasitoid?

Parasitoids are parasitic endopterygote insects (undergoing full metamorphosis – egg, larva, pupa, adult – with wings developing internally) where the adult is free living while the larvae develop by feeding on or within arthropod hosts. A single host individual sustains the development of one or more parasitoids, and the host is almost invariably killed by the interaction (Mills 1994; Quicke 1997). A parasite, on the other hand, is defined as an organism that for all or some part of its life derives its food from a living organism of another species, and the host is generally harmed to some extent but is usually not killed by the association.

In the older literature, parasitoids are often referred to simply as "parasites", which could cause confusion. Several alternative terminologies have been proposed, and in recent years "parasitoid" has become the generally accepted term. This grouping is not a monophyletic entity since parasitoids are known from seven insect orders (reviewed by Quicke 1997). However, the vast majority of all parasitoid species belong to the order Hymenoptera which comprise a highly successful group with probably more than a million species (Quicke 1997), or about 20% of all insect species (LaSalle and Gauld 1991). "Parasitic hymenopteran" does, with few exceptions, imply the same thing as "parasitic wasp", and both terms are used widely in recent literature. Both terms are used in this thesis. Herein, "parasitoid" is used synonymously with "Hymenopteran parasitoid", as this is the order addressed in my research.

#### Parasitoid Species Addressed

This thesis concerns four of the most common parasitoid species associated with the bark beetle *Ips typographus*, although these parasitoids are polyphagous and attack other bark beetle species as well. The parasitoids originated from Sweden and southeastern USA. Their potential as control agents against *Ips* and *Dendroctonus* beetles has been evaluated by Mills and Schlup (1989). The family Braconidae, of the superfamily Ichneumonoidea (Hedqvist 1998), contains the largest parasitoids (ca 10 mm), while the smaller species (ca 3–6 mm) belong to the Pteromalidae, of the superfamily Chalcidoidea (Bouček and Heydon 1997; Grissell and Schauff 1997).



◆ *Coeloides bostrichorum* Giraud (Hymenoptera: Braconidae) (from Sweden: Paper V) is mainly distributed in northern and central Europe, eastern European Russia, western Siberia, and Mongolia (Tobias et al. 1986; Krüger and Mills 1990). Oviposition is conducted through the bark. The ovipositor of *C. bostrichorum* is longer than those of the other studied parasitoids, which makes it possible for this species to reach hosts at greater depth. *Coeloides bostrichorum* has been reported as the most efficient bark beetle parasitoid of *I. typographus* (Krüger and Mills 1990). This species seems to be entirely confined to bark beetles breeding in spruce (Hedqvist 1998).

◆ *Rhopalicus tutela* (Walker) (Hymenoptera: Pteromalidae) (from Sweden: Papers I, II, and VI) is widely distributed in Europe (Graham 1969) and North America (Burks 1979). It is one of the more aggressive species, fighting off other parasitoids at oviposition sites on the bark surface (Krüger and Mills 1990). Oviposition is conducted through the bark. This species has been reported as an important parasitoid of the bark beetles *Ips acuminatus* (Gyllenhal) and *Tomicus piniperda* (L.) (Herard and Mercadier 1996).

◆ *Roptrocerus mirus* (Walker) (Hymenoptera: Pteromalidae) (from Sweden: Paper II) is the smallest of the studied parasitoids. This species is reported from Europe (Graham 1969; M. Kenis, personal communication) and Asia (Yang 1987). *Roptrocerus* spp. enters the bark beetle galleries to oviposit.

◆ *Roptrocerus xylophagorum* (Ratzeburg) (Hymenoptera: Pteromalidae) (from Sweden: Paper II, and from southeastern USA: Papers III and IV) has been found in parts of Europe, America, and Asia (Graham 1969; Burks 1979) where it is native. It has been successfully introduced against exotic *Ips grandicollis* bark beetles in Australia. *Roptrocerus xylophagorum* has been reported as an important parasitoid of the bark beetles *I. grandicollis* and *Dendroctonus frontalis* (Moore 1972; Mills 1983). Several other economically important scolytids are also parasitised successfully by this species (Mills 1983). *Roptrocerus xylophagorum* responds quickly to changes in host numbers (Dahlsten 1982), hence it may be an effective regulator of bark beetle populations.

## Attack Strategies

Parasitoids of bark beetles can attack various developmental stages of their host. However, all of the parasitoids studied in this thesis are solitary parasitoids, which primarily attack late instar bark beetle larvae. This is the preferred host stage for most parasitoids preying on bark beetles (Dahlsten 1982). These late instars are present in larval mines or



pupal chambers under the bark of coniferous host trees, where the bark beetles reproduce. In addition, *R. tutela* (Krüger and Mills 1990) and *R. xylophagorum* (Samson 1984) have been reported to occasionally oviposit on young pupae.

In addition to attacking different hosts or host stages, hymenopteran parasitoids also employ a variety of attack strategies. The idiobiont strategy is an attack strategy that comprises several parasitoid life-history characteristics, some are given in Table 1. The parasitoids discussed in this thesis are all classified as idiobionts.

<b>Idiobiont characteristics</b>	
Generalists	attacking several host species
Host concealed	hosts typically embedded in plant tissue, such as bark beetle larvae present in pupal chambers beneath the bark of the host tree
Ectoparasitism	laying their eggs onto or close beside the host, parasitoid larvae feed externally on the host
Long-term host paralysis	female parasitoids paralyse the host before ovipositing
Host stage attacked larger than the wasp	
Relatively long adult life span	
(from about 20 days to several months, Krüger and Mills, 1990)	

**Table 1.** Some traits associated with idiobiont parasitoid strategy (Quicke 1997).

## The Life of a Bark Beetle Parasitoid

Although bark beetle parasitoids are free-living during most of their life, they require a bark beetle host (e.g. a mature bark beetle larva) to complete their reproductive cycle. Parasitoid females locate susceptible hosts, which are paralysed with a venom injection prior to oviposition (egg laying) (Quicke 1997). To avoid superparasitism, the female parasitoid may mark the host/oviposition site (Lenteren 1981). During its development the parasitoid larva kills the bark beetle larva.

The development of parasitoids is very temperature dependent (Krüger and Mills 1990). Parasitoids can overwinter beneath the bark as immature stages. Some species are reported to enter diapause (mainly below 15°C) while others do not have a true diapause and become active again with warm temperatures (Krüger and Mills 1990). At temperatures above 18°C parasitoids typically emerge as adults 3–4 weeks after oviposition. Male

parasitoids emerge ahead of females, and aggressively compete for females that appear later on the bark surface. *Rhopalicus tutela* females have a number of mature eggs in their ovaries already when they emerge, and can start host searching immediately after mating. However, *C. bostrichorum* females requires a pre-oviposition period of 8–10 days, at 20°C, after mating to develop mature eggs (Krüger and Mills 1990).

Hymenopterans have haplodiploid sex determination, in which unmated females lay unfertilised (haploid) eggs that develop into males, while mated females lay fertilised (diploid) eggs that develop into females (Quicke 1997). Other factors affecting the sex ratio in hymenopteran parasitoids are host age, maternal age and nutritional status, host density, and parasitoid density (Waage 1982; Waage et al. 1985; King 1987). In addition, many parasitoids are able to distinguish hosts of different size laying unfertilised, male, eggs on smaller hosts and fertilised, female, eggs on larger hosts (Kenis 1996). A convincing explanation for this strategy is that parasitoid fitness should increase with increased parasitoid size, which in turn increases with host size, and that females gain more from being large than males do (Charnov et al. 1981). It is not yet known what cues the female parasitoids use to determine host suitability.

Many hymenopteran parasitoid species play a valuable role in pest control, and much of the interest in parasitoid biology is due to an interest in improving biological control (i.e. importation, augmentation and conservation) through a better understanding of the control agents (Vinson et al. 1998). The first documented use of a parasitoid in biological control involves the importation and release of the dipteran *Cryptochetum iceryae* (Williston) from Australia in 1888 against cottony cushion scale in California. This parasitoid established successfully and still contributes in controlling the host populations (Mills 1994). However, the efficacy of parasitoids in regulating host population density is influenced by many factors (as mentioned above), therefore knowledge on their biology is valuable for biological control programs. Furthermore, parasitoids are essential in maintaining the diversity of natural communities (Quicke 1997). Parasitoids' high position in the food chains suggests that they are particularly liable to extinction and also likely to include many keystone species (LaSalle and Gauld 1991).

# Bark Beetles

## Bark Beetle Species Addressed

There are at least 6,000 bark beetle species from 181 genera worldwide (Byers 1995). In Sweden there are 82 species, and about 10 breed in trees of economic significance (Spessivtseff 1922; M. Knížek, personal communication). All of the three bark beetle species (Coleoptera: Scolytidae) examined in this study breed in conifers (Pinopsida: Pinaceae).

♦ *Ips typographus* L. (the eight spined spruce bark beetle, from Sweden: Papers I, II, V, and VI) breeding in Norway spruce (*Picea abies* L. Karst.). *Ips typographus* belongs to one of the most aggressive bark beetle genera (Mills 1991), killing healthy trees during epidemic outbreaks. This beetle causes damage to spruce forests and is of economical importance in Swedish forestry.

♦ *Ips grandicollis* Eichhoff (the southern pine engraver, from southeastern USA: Papers III and IV) breeding in loblolly pine (*Pinus taeda* L.). Although not as aggressive as the other two species, *I. grandicollis* has caused damage to Australian pine forests, where it was accidentally introduced and lacked native natural enemies (Samson and Smibert 1986; Berisford and Dahlsten 1989; Abbott 1993).

♦ *Dendroctonus frontalis* Zimmermann (the southern pine beetle, from southeastern USA: Papers III and IV) breeding in loblolly pine. This is the most destructive forest pest in the southern United States and an obligate tree killing species (Payne 1980).

## The Life of a Bark Beetle

Semiochemicals, both from trees and bark beetles, are crucial for bark beetle reproduction. The resin of conifers is both a physical (sticky) and a chemical (toxic) barrier, protecting the tree against bark and wood boring insects. To overcome this defence of their host trees, the first colonising bark beetles co-ordinate a massive attack by emitting aggregation pheromones that attract conspecifics of both sexes. The beetles excavate galleries in the host tree phloem, where they reproduce. During this process various associated microorganisms, particularly yeasts and fungi (Leufvén and Birgersson 1987, Krokene and Solheim 1996), are introduced into the phloem. The trees are killed by beetle mining that girdles the phloem, and the growth of associated microorganisms, that cut off the water and nutrient transportation in the vascular tissues of the tree. Microorganisms seem to hasten tree death although *D. frontalis* is able to



kill trees in the absence of microorganisms (Bridges et al. 1985). Bark beetle females lay eggs, hatching larvae generate galleries, and late larval stages excavate pupal chambers where they pupate. About 40 days after infestation (by *I. typographus* under laboratory conditions, at 20°C) the new generation of bark beetles emerge from the host tree.

## Parasitoid-Host Interactions

### Host Location Mechanisms

Host location by parasitoids is a complex process that typically can be divided into five main steps: host habitat location (i.e. long-range host location), followed by short-range host location that in turn is divided into host location, host acceptance, host suitability and host regulation (Vinson et al. 1998). It is generally recognised that host location likely involves multiple cues and several sensory modalities (De Moraes and Mescher 1999). Concerning bark beetle parasitoids, however, cues such as infrared radiation (heat), and host generated vibrations have been ruled out (Richerson and Borden 1972; Mills et al. 1991; Paper I). Semiochemical\* stimuli are commonly used by host foraging hymenopteran parasitoids (Vinson 1985; Rutledge 1996; Vinson et al. 1998).

### Multitrophic Interactions

Parasitoids attacking larvae commonly function within a tritrophic context (parasitoid–host–host habitat). Host foraging parasitoids have to deal with the so-called detectability/reliability problem (Vet et al. 1995). This means that although the signals emitted by the hosts are very reliable indicators of a true host, such signals are difficult to detect as the hosts are under selection to avoid being found and parasitised. On the other hand, signals from the host substrate (e.g. a spruce tree), on which the hosts feed or breed, should be easy to detect but might not necessarily indicate the presence of susceptible hosts. Consequently, parasitoids that attack bark beetle larvae are under continuous selection pressure to optimise their host foraging strategy, since efficient location of well hidden, and often scarce, hosts is critical for successful reproduction.

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\* "Semiochemical", or the synonymous "infochemical", is the general term for any chemical that is emitted by one organism and invoking a physiological or behavioural response in another organism. For terminology see Nordlund and Lewis (1976) or Dicke et al. (1990).



To optimise their host foraging, the parasitoids often use cues both from the host and its habitat (e.g. Vet et al. 1995; Rutledge 1996; De Moraes and Mescher 1999). Parasitoids with prior experience of host related cues are able to increase their host foraging efficacy. Furthermore, they are also able to learn additional cues indirectly associated with the presence of susceptible hosts (Vet et al. 1995). There are numerous examples of multitrophic communication systems, where host foraging parasitoids use volatile cues from various origins and trophic levels (reviewed by Rutledge 1996). For bark beetle parasitoids, such cues may be emitted by the host insect itself, its products (e.g. boring dust or faeces), the host tree, or host-associated microorganisms (Dixon and Payne 1980; Krokene and Solheim 1996). Thus, parasitoids of bark beetles function within a multitrophic context. Besides the parasitoid itself, this multitrophic interaction involves the coniferous tree, the bark beetle larvae, and the bark beetle associated microorganisms that are introduced into the host tree phloem.

Natural selection is expected to favour parasitoids that are most efficient in host foraging, and bark beetles are under selection pressure to avoid being found and parasitised. Thus, the major cues used in host foraging are expected to originate not directly from the host organism itself but from other host associated sources. There are numerous examples showing how the feeding by phytophagous insects induces qualitative and quantitative changes in the volatile composition of plants, which attract parasitoids of (e.g. Turlings et al. 1991; Rutledge 1996). Alborn et al. (1997) showed that a specific compound in the oral secretions of a phytophagous insect induces such plant emissions. In the bark beetle parasitoid interaction, the tree is dying or is already dead. However, dead host habitat material, with the hosts removed, may still emit volatiles attracting parasitoids (Takács et al. 1997).

## Methodological Considerations

### Experimental Insects & Host Trees

Norway spruce logs cut from 50–90 years old trees infested with parasitised bark beetle larvae were collected in forests of southern Sweden during late summers (mainly in the province of Småland). The logs were treated and stored for later use as described in detail in Paper I. Parasitoids and bark beetles to be used in the experiments were reared out from these logs. Un-infested logs were also collected, to be used as such

(fresh logs) or to be infested by bark beetles in the laboratory. Parasitoids from southeastern USA (Papers **III** and **IV**) were obtained from a laboratory colony. These insects were reared continuously on freshly cut logs of loblolly pine infested in the laboratory with *I. grandicollis* (Sullivan et al. 1999).

## Summary of Techniques

The analytical techniques used in this thesis are listed below, with references to the papers that give further details:

- ◆ The antennal morphology of the parasitoids was examined by Scanning and Transmission Electron Microscopy (SEM and TEM) (Paper **I**).
- ◆ The possible presence of "hot spots" on the bark surface above pupal chambers containing bark beetle larvae was investigated with an Infrared Radiation Scanner (Paper **I**).
- ◆ Volatiles were collected from logs or pieces of bark containing susceptible bark beetle hosts, in order to isolate potential host location cues. Host associated volatiles were collected from various bark beetle and host tree species. Both whole logs, isolated larvae, and pupal chambers were sampled. Three collection techniques were used: Dynamic Headspace Adsorptions (Papers **II**, **IV**, **V**, and **VI**); Static Headspace Solid Phase Micro-Extraction (HS-SPME) (Paper **VI**); and Steam-Distillation (Papers **III** and **IV**).
- ◆ Electrophysiological analysis by combined gas chromatography-electroantennographic detection (GC-EAD) was performed to screen for compounds in the volatile collections that elicited neural activity in the parasitoid antennae, thus being potential host location cues (Papers **I**, **II**, **III**, **V**, and **VI**). Some of these GC-EAD active compounds were then used to prepare synthetic baits for behavioural assays.
- ◆ Compounds present in the odour samples were analysed and identified by combined GC-mass spectrometry (GC-MS). Identification of GC-EAD active compounds received special attention, since these are potential host location cues (Papers **I–VI**).
- ◆ The attraction of various species of female and male parasitoids to natural odour sources (bark or logs with or without bark beetle larvae) and synthetic baits was examined with two types of bioassays: Short range attraction of walking parasitoids was tested in a Y-tube olfactometer (Papers **II**, **III**, and **IV**), and long range attraction of flying parasitoids was assayed in a wind tunnel (Paper **V**).

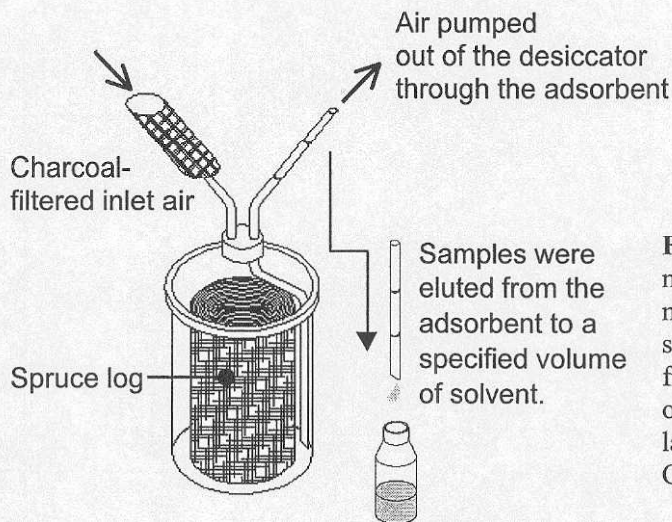
## Sampling of Volatile Compounds

Chemical Ecology research requires methods for sampling volatile chemicals (odours) from biological material. Sampling of volatile compounds in the headspace (the air surrounding the sampled material) can be performed in several ways. A variety of adsorption filters (adsorbents) are commercially available for sampling of different types of volatile compounds. Since compounds of interest often are released in minute amounts into the surrounding air, sampling generally requires some sort of enrichment. Such enrichment is typically done by first enclosing the material that is to be sampled, within a limited volume, thus increasing the concentration of compounds in the headspace and minimising the introduction of contaminants. Sampling can then be achieved by introducing an adsorbent, such as a Solid Phase Microextraction fiber (SPME, Pawliszyn 1997), into the fixed volume of headspace (static headspace). Alternatively, very clean air can be allowed to pass over the sample and through an adsorbent (dynamic headspace adsorption). The adsorbents can then either be desorbed thermally directly in the GC injector, or extracted with a solvent. The latter desorption method has the advantage that samples can be analysed repeatedly and can be concentrated by partially evaporating the solvent. The different sampling methods that are available have recently been reviewed and discussed by Millar and Sims (1998).

Techniques used in this thesis to sample volatile compounds:

- ◆ Dynamic headspace adsorption was used to collect volatiles from whole spruce logs, with or without bark beetle hosts (Papers II and V). Single logs were enclosed in a glass desiccator, Figure 1, and air was drawn through the enclosure onto a Porapak Q (divinyl benzene-ethylvinylbenzene, a slightly polar copolymer) adsorbent (Alltech). Porapak Q was chosen as adsorbent due to its trapping capacity of semipolar compounds, such as oxygenated monoterpenes (the compounds of main interest in this thesis) and other conifer related compounds. The sampled volatile compounds were eluted from the adsorbents with diethyl ether. Volatiles were also collected from pieces of infested or un-infested bark (Paper IV), and at close-range from pupal chambers, with or without mature bark beetle larvae, as well as from larvae outside the pupal chambers (Paper VI).





**Figure 1.** The equipment used for dynamic headspace adsorption of volatiles from spruce logs with or without bark beetle larvae (illustration by Claes Johansson).

◆ Solid Phase Microextraction (SPME), with carbowax-divinylbenzene as the adsorbing phase, was used for sampling the headspace, in an enclosed volume, over pupal chambers with or without bark beetle larvae (Paper VI). This particular fiber phase was chosen since it is efficient in trapping semipolar compounds (e.g. oxygenated monoterpenes). Some of the advantages and disadvantages of sampling by SPME from this material are discussed in Paper VI.

◆ Steam-Distillations of pieces of bark containing susceptible hosts were performed as described by Sullivan (1997) and Sullivan et al. (1997) to collect odours associated with the host species from the USA (Papers III and IV). Such odours were also collected by dynamic headspace adsorption (Paper IV).

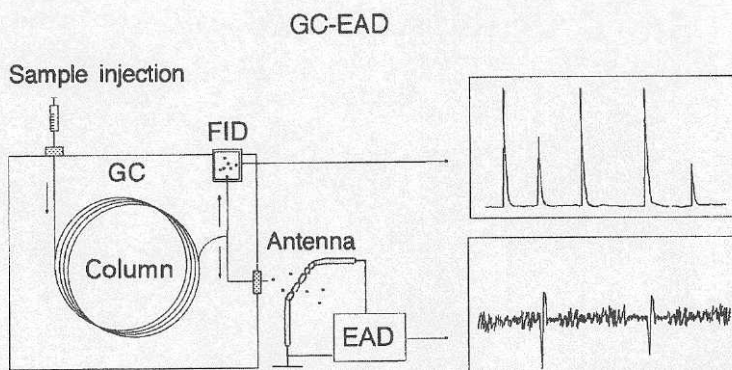
## Analysis of Odour Samples

### *Electrophysiology*

Combined gas chromatography-electroantennographic detection, GC-EAD, was used to screen for compounds eliciting neural activity in the parasitoid antenna. Electroantennographic methods (GC-EAD and electroantennography, EAG) measure the summed neural activity of all sensilla (small sensory structures) present on the antenna. In the schematic GC-EAD recording in Figure 2, two out of five compounds elicit neural activity in a parasitoid antenna, which is mounted between a grounded and a recording electrode measuring changes in potential over the antenna (Arn et al. 1975). Each insect was recorded only once. To ensure that lower response magnitudes at the end of the recording period



were not due to weakened antennae, the condition of the antennae was tested by EAG before and after each GC-EAD recording (Fig. 2\* in Paper III). In each EAG test, the antenna was exposed to a standard blend of volatiles that was not separated by GC. The blend in a Pasteur pipette was puffed into the glass interface between the GC exit and insect antenna.



**Figure 2.** GC-EAD analysis, with simultaneous recordings of signals from a GC equipped with a flame ionisation detector (FID), and an insect antenna connected to an electroantennographic detector (EAD). The injected sample is split (1:1) between the two detectors (modified from Bäckman 1999).

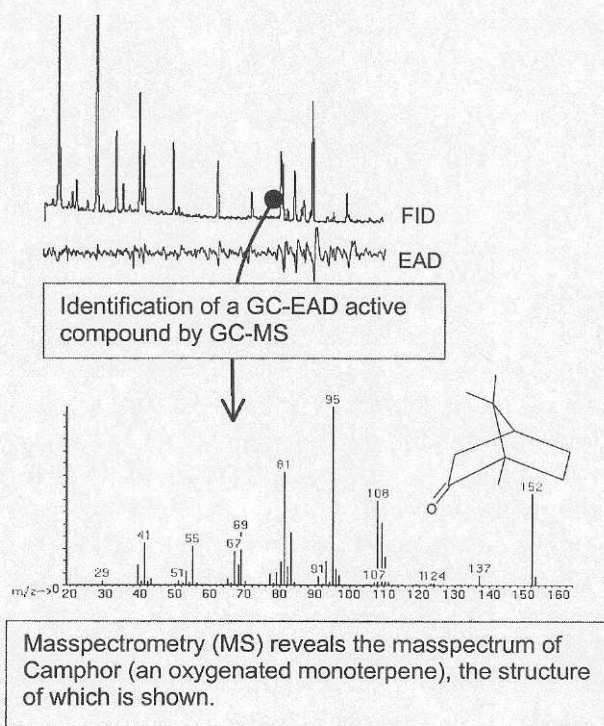
### *Chemo-Electrical Signal Transduction*

The minute openings (pores) in the cuticular walls of chemoreceptive sensilla (Figure 4) allow chemicals to diffuse into the sensillar lumen. In the lumen, there are odourant binding proteins, which may facilitate the transfer of hydrophobic odourant molecules to the sensory dendrite although a definite proof for the function is lacking (Breer 1997). There are specific odourant binding sites, receptors, residing on the dendritic membrane of olfactory sensory neurones. Several similar types of molecules can bind to the same site, although certain molecules can generate a stronger response than others do. The binding of odourants to the receptors trigger a chain-reaction, resulting in the activation of ion channels ( $\text{Ca}^{2+}$  or  $\text{K}^+$ ) in the dendritic membrane. This, in turn, generates an electrical impulse (depolarisation) over the membrane, which is propagated to the central nervous system and registered as a response (reviewed by Breer 1997; Krieger and Breer 1999). Electrical impulses can be detected by electrophysiological techniques, enabling a screening of potentially behaviourally active compounds.

\* A Figure in a Paper is referred to as "Fig.", while a figure in the thesis is referred to as "Figure".

### Chemical analysis

Combined gas chromatography-mass spectrometry (GC-MS) identified the compounds present in the collected volatile samples. Semipolar, polyethylene glycol (PEG) coated columns (Cp-Wax 58 CB, or HP Innowax) were used both in GC-EAD and GC-MS analyses, facilitating identification of antennal active compounds. These column phases were used since they efficiently separate semipolar compounds, such as oxygenated monoterpenes associated with damaged conifers. Compounds were identified by their mass spectral matches to library spectra and by retention time matches to known standards.



**Figure 3.** Chemical analysis of volatile samples and identification of GC-EAD active compounds were done by GC-MS.

## - MAIN RESULTS AND IMPLICATIONS -

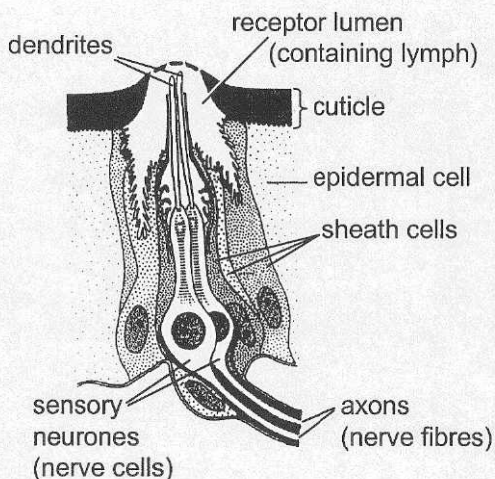
My results imply that odours are the most important host location cues for parasitoids that attack bark beetles breeding in conifers. Synthetic attractants for the addressed parasitoids were developed. A limited number of key compounds are required for host location although the volatile compositions of host infested materials is complex. Specific oxygenated monoterpenes have been identified as essential host foraging cues, both in Swedish and North American parasitoids, irrespective of bark beetle host and coniferous tree species. Some of these compounds increase in concentration as bark beetle larvae develop. Compounds attracting parasitoids do not arise directly from bark beetle larvae but from the interaction of the bark beetle, its associated microorganisms, and the infested coniferous host tree tissue.

### The Role of the Antennae

#### Antennal Morphology

Electron microscopy (SEM and TEM) were used to elucidate which sensillar types (sensory modalities) were present and most abundant on the antennae of *R. tutela* (Paper I). Olfactory placoid, tricoid, and coeloconic sensilla were found to be abundant on the antennae of both females and males, implying the importance of odour perception. Placoid sensilla were the most abundant type of sensilla. A view of a schematic placoid sensillum is shown in Figure 4, to be compared with the SEM and TEM photographs of this sensillum from *R. tutela* in Paper I (Figs. 2 and 3). Amputation experiments have shown that parasitoid females failed in locating bark beetle larvae if less than half of the parasitoid antennae were left (Richerson and Borden 1972). This indicates that the antennae are necessary in host finding, although sensilla can be found on several other parts of the insect body (e.g. mouthparts, tarsi, and ovipositor) (Slifer 1970). Based on structural evidence, it is possible to state if a sensillum is, or is not, a chemoreceptor (Slifer 1970).





**Figure 4.** Transverse section through a schematic *Sensillum placodium* with porous walls, which is a typical feature of a chemoreceptor. The space beneath the porous cuticle (lumen) is filled with dendrites, among which two are shown in the figure (modified from Gullan and Cranston 1994).

## Host Location Cues

Parasitoids face a serious challenge in detecting their concealed bark beetle hosts, with no obvious visual cues available. It is plausible that cues other than odours are complementary in host foraging. Previous studies on the antennal morphology of the bark beetle parasitoid *Coeloides brunneri* (Braconidae) suggested infrared-perceptive abilities of sensilla placodea, since no cuticular pores were detected (Richerson et al. 1972). However, in Paper I cuticular pores were found in this type of sensilla, and no "hot spots" (increased IR-radiation) could be detected above pupal chambers (Paper I), supporting the results by Mills et al. (1991) that reject infrared radiation as a host location cue. Numerous studies show that parasitoids are attracted to volatiles associated with their hosts (reviewed by Rutledge 1996), although information on host location cues in bark beetle parasitoids is scarce. The results from Paper I, provide evidence for the importance of olfactory cues in bark beetle parasitoids, and thus constitute the basis for the rest of the work in this thesis.

## Chemical Cues of Hidden Hosts

### Antennal Activity

There is no previous report on the use of GC-EAD technique on bark beetle parasitoids. Therefore, the work of this thesis started by investigating the odour perceptive abilities of the parasitoid antennae by assaying a synthetic standard blend with GC-EAD (Paper I). Both female and male *R. tutela* were analysed (15 individuals of each gender). To

measure the reliability of the GC-EAD response to a certain compound, this was expressed as the mean  $\pm$  standard error. By measuring several individuals exposed to the same stimuli, it was possible to quantitatively evaluate the response elicited by a defined amount of a certain compound.

Paper I showed that certain host related compounds elicit neural activity of reproducible magnitude in antennae from different individuals. There were no differences in responsiveness between female and male *R. tutela*. Monoterpene hydrocarbons (constitutive compounds of fresh host-trees) did not elicit detectable EAD-responses. All antennal active compounds were either bark beetle pheromone components or oxygenated monoterpenes. The conclusion of Paper I is that the antennae of *R. tutela* are well adapted to odour perception, and that certain host related odours elicit neural activity in the antennae.

### Volatiles from Host Infested Logs

Based on the results from Paper I, the thesis work proceeded by investigating volatiles sampled from conifer logs containing parasitism-susceptible bark beetle larvae (Figure 1). GC-EAD analyses (Figure 2) were performed to isolate compounds eliciting neural activity, thus being potential host location cues.

A summary of all GC-EAD active compounds found in the tested odour samples and standard mixtures are given in Table 2. Swedish *R. xylophagorum* has been found to respond similarly as the tested Swedish parasitoids when exposed to odours collected from whole logs (as in Paper II) or pupal chambers (as in Paper VI) (E.M. Pettersson, unpublished data). The GC-EAD active compounds were mainly oxygenated monoterpenes (indicating damaged conifers) in all investigated species, irrespective of bark beetle host and coniferous tree species.

Female *Rhopalicus tutela* and *Roptrocerus mirus* revealed similar trends in responsiveness to host associated volatiles. Most monoterpene hydrocarbons (tree compounds) were not antennal active, in contrast to several oxygenated monoterpenes. Camphor (co-eluting with minor quantities of pinocamphone) and isopinocamphone elicited the strongest antennal activities (Paper II, Fig. 2). Similar results were obtained from *Coeloides bostrichorum* (Paper V, Fig. 1). *Roptrocerus xylophagorum*, originating from southeastern USA and exposed to volatiles associated with bark beetles breeding in loblolly pine, revealed slightly different trends in responsiveness (Paper III, Fig. 3). However, the differences in responsiveness are most probably due to different relative abundance of

compounds in samples collected from Norway spruce (Sweden) or from loblolly pine (USA).

**Table 2.** Compounds eliciting EAD-activity in parasitoids attacking bark beetle larvae. Highlighted compounds were used in preparing various synthetic baits tested in bioassays. (The footnote indicates the compounds used in behavioural tests with each parasitoid species. The specific compositions of the various baits are given in the specified papers.)

Compound	Species addressed			Papers
	Parasitoid	Bark beetle	Tree	
Acetic acid	Cb	It	Pa	V
4-Allylanisole	Cb; Rt; Rm; Rx	Df; Ig; It	Pa; Pt	II; III; V
Anethol <sup>1</sup>	Rx			III
Benzaldehyde	Cb; Rt	It	Pa	V; VI
Borneol <sup>e</sup>	Cb; Rt; Rm; Rx <sup>1</sup>	It	Pa;	I; II; III; V
Bornyl acetate	Rt <sup>2</sup> ; Rx <sup>1</sup>			I; III
C <sub>3</sub> -benzene	Cb	It	Pa	V
C <sub>4</sub> -benzene	Cb	It	Pa	V
C <sub>4</sub> -styrene	Rx	Df; Ig	Pt	III
δ-Cadinene	Rt	It	Pa	VI
Camphor <sup>a, b, c, d, e</sup>	Cb; Rt; Rm; Rx	Df; Ig; It	Pa; Pt	I; II; III; V; VI
<i>cis</i> -Chalcogran <sup>2</sup>	Rt			I
<i>trans</i> -Chalcogran <sup>2</sup>	Rt			I
<i>p</i> -Cymen-8-ol	Rt	It	Pa	VI
<i>p</i> -Cymene	Rt; Rm	It	Pa	II; VI
Fenchol	Rt; Rx	Df; Ig; It	Pa; Pt	III; VI
Fenchone <sup>a, b, c, d, e</sup>	Cb; Rt; Rm; Rx	Df; Ig; It	Pa; Pt	I; II; III; V; VI
Heptanol	Cb	It	Pa	V
Ipsdienol <sup>2</sup>	Rt			I
Ipsenol <sup>2</sup>	Rt			I
Isopinocampnone <sup>a, b, c, d, e</sup>	Cb; Rt; Rm; Rx <sup>1</sup>	It	Pa	I; II; III; V; VI
Limonene	Rt	It	Pa	VI
Linalool	Rx	Df; Ig	Pt	III
Myrtenal <sup>e</sup>	Cb; Rt; Rm; Rx	Df; Ig; It	Pa; Pt	II; III; V; VI



Table 2. (continuing)

Compound	Species addressed			Papers
	Parasitoid	Bark beetle	Tree	
Myrtenol <sup>a, d</sup>	Cb; Rt; Rx	Df; It	Pa; Pt	I; II; III; V; VI
Nonanal	Cb	It	Pa	V
$\beta$ -Phellandrene	Rt	It	Pa	VI
Pinocamphone *	Rt; Rm	It	Pa	II; VI
<i>trans</i> -Pinocarveol <sup>a, b, c, d</sup>	Cb; Rt; Rm; Rx	Df; Ig; It	Pa; Pt	I; II; III; V; VI
Pinocarvone	Rt; Rm; Rx	Df; It	Pa; Pt	II; III; VI
Terpien-4-ol <sup>a, b, c, d, e</sup>	Cb; Rt; Rm; Rx	Df; Ig; It	Pa; Pt	I; II; III; V; VI
$\alpha$ -Terpineol <sup>a, d, e</sup>	Cb; Rt; Rm; Rx	Df; Ig; It	Pa; Pt	II; III; V; VI
Terpinolene	Cb; Rt; Rm	It	Pa	II; V; VI
u.i. 83; 152	Rt; Rm	It	Pa	II; VI
u.i. 79; 93; 137	Rx	Df; Ig	Pt	III
u.i. 81; 93; 107	Cb	It	Pa	V
u.i. 83; 55; 95	Cb; Rt; Rm	It	Pa	II; V
u.i. SqT	Cb; Rt; Rx	Ig; It	Pa; Pt	III; V; VI
<i>trans</i> -Verbenol <sup>2</sup>	Rt			I
Verbenone <sup>a, d</sup>	Cb; Rt; Rm; Rx	Df; It	Pa; Pt	I; II; III; V; VI

u.i. = un-identified compound, predominant mass spectral fragments are given; SqT = sesquiterpenes; Cb = *Coeloides bostrichorum* (Sweden); Rt = *Rhopalicus tutela* (Sweden); Rm = *Roptrocerus mirus* (Sweden); Rx = *Roptrocerus xylophagorum* (USA); Df = *Dendroctonus frontalis* (USA); Ig = *Ips grandicollis* (USA); It = *Ips typographus* (Sweden); Pa = *Picea abies* (Sweden); Pt = *Pinus taeda* (USA)

<sup>1</sup> Constituent of the synthetic blend analysed in Paper III, where the origin of compounds is given.

<sup>2</sup> Constituent of the synthetic blend analysed in Paper I, where the origin of compounds is given.

\* Pinocamphone co-eluted with camphor, and was not measured separately.

<sup>a-e</sup> A component of at least one of the synthetic baits used in behavioural tests with a specified parasitoid species. The specific compositions of the various synthetic baits are given in the respective papers:

<sup>a</sup> Swedish *Coeloides bostrichorum* (Paper V).

<sup>b</sup> Swedish *Rhopalicus tutela* (Paper II).

<sup>c</sup> Swedish *Roptrocerus mirus* (Paper II).

<sup>d</sup> Swedish *Roptrocerus xylophagorum* (Paper II).

<sup>e</sup> North American *Roptrocerus xylophagorum* (Paper III).

The addressed parasitoid species seem to have the same odour perceptive abilities, since the over all responses of different parasitoid species to host related compounds are very similar. However, the natural baits used in the different GC-EAD analyses were collected from different host materials, therefore there were some qualitative and quantitative differences between these baits. Differences in the bait compositions are the most probable reason why compounds occasionally elicited responses in some parasitoids but not in others.

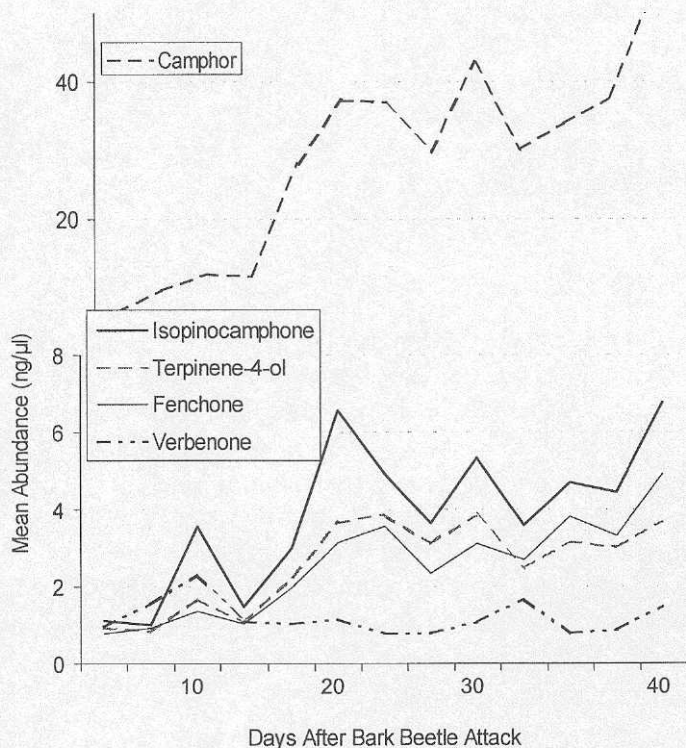
## Behavioural Attractants

Electrophysiological methods isolate compounds that elicit neural activity, however it does not disclose the behavioural significance of these compounds. This issue requires behavioural assays (bioassays), which were used to test attraction of walking or flying parasitoids to various volatile baits. The attraction of parasitoids to "natural baits" (i.e. logs or pieces of bark, with or without susceptible hosts) and "synthetic baits" (composed of compounds associated with susceptible hosts) were assayed in behavioural tests. A limited number of oxygenated monoterpenes was required to attract female parasitoids.

### Baits in Bioassays

- ◆ Natural baits tested in bioassays were either whole logs (Paper II), dynamic headspace adsorptions from such logs (Paper V), pieces of bark, isolated hosts (Paper IV), or steam distillates from pieces of bark (Papers III and IV). The chemical compositions of the natural baits used were analysed, and are presented in the respective paper.
- ◆ Synthetic baits (Papers II, III, and V) were composed of compounds in the natural baits that elicited GC-EAD activity (Table 2), as identified by GC-MS (Figure 3). In most synthetic baits the proportions among the compounds reflected their abundance in the natural baits. The compositions of the various synthetic baits are given in the respective papers. Some of the synthetic baits (Papers II and V) were partly based on chemical analysis of changes in the volatile compositions of six Norway spruce logs during bark beetle infestation, until young bark beetles emerged (E.M. Pettersson, unpublished data). Corresponding reference analyses were done of un-infested logs. Some GC-EAD active compounds, as camphor and isopinocampone, were found to increase quantitatively as bark beetle larvae developed (Figure 5). Verbenone was not found to increase significantly after three weeks but was used in

synthetic baits (Papers II and V) based on its relatively strong GC-EAD activity.



**Figure 5.** The abundance of certain oxygenated monoterpenes increase as bark beetle larvae develop (ca 20 days after a bark beetle attack).

In nature, parasitoids are attracted to conifers containing susceptible bark beetle larvae. These differ chemically from un-infested trees by having higher concentrations of oxygenated monoterpenes. However, there were no compounds detected in un-infested trees that are not also present in infested trees. One aim when bioassaying synthetic baits was to find the least number of compounds required to elicit attraction. Therefore, in the behavioural tests of Paper II (Fig. 1) and Paper V (Figure 6), odours from an un-infested spruce log were used in combination with various synthetic baits (simulating an infested tree). Synthetic baits were tested alone in Papers III and V.

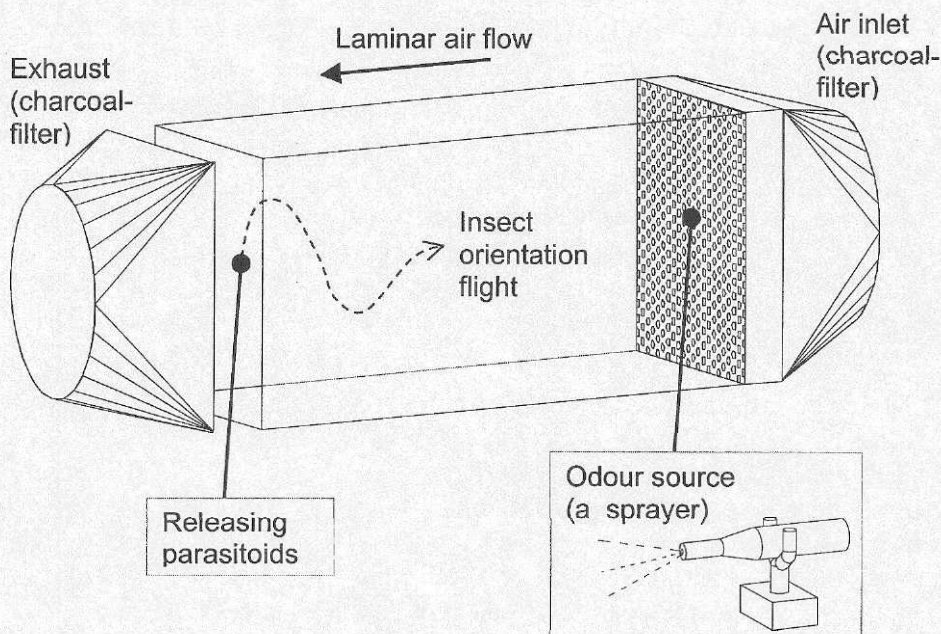
### Assaying Attractants

The first step in bioassays was to observe the attraction to natural baits obtained from bark beetle infested trees, versus natural baits from un-infested trees or solvents. Parasitoid females significantly preferred volatiles from bark beetle infested trees (Papers II, III, IV, and V). In contrast, male parasitoids did not show any such preference (Papers II



and III). The female responses were presumably related to host location, while the absence of male responses was expected since males derive no obvious benefit from attraction to bark beetle larvae.

Synthetic baits, composed of 3–8 compounds, were found to be attractive when tested against a solvent blank or un-infested conifers in Y-tube walking bioassays with female *R. tutela*, *R. mirus*, and *R. xylophagorum* (Papers II and III). In the wind tunnel (Figure 6), female *C. bostrichorum* oriented towards synthetic baits composed of at least three compounds (Paper V, Fig. 2). Synthetic baits were not as attractive as bark (Paper III) or spruce logs (Paper V) containing susceptible hosts. However, when certain synthetic baits were combined with odours from un-infested spruce logs (which were not attractive alone), this combination was as attractive as infested logs (Papers II and V). These results show that a limited number of key compounds are required to attract parasitoids. The oxygenated monoterpenes camphor, isopinocamphe, and terpinen-4-ol seem to be among the most important compounds.



**Figure 6.** Experimental equipment for wind tunnel bioassays (illustration by Claes Johansson).

## Possible Sources of Parasitoid Attractants

The aims of this thesis were not only to isolate and identify potential host location cues, but also to isolate the source emitting these parasitoid attractants. The results show that the compounds attracting parasitoids do not primarily arise from the bark beetle hosts but rather from the interaction of the bark beetle, its associated microorganisms, and the phloem and cambium tissue of the host plant (Papers IV and VI). Host location cues that originate directly from the bodies of the host insect is probably quite rare in nature. As mentioned in the "Multitrophic Interactions" section, there are instead numerous examples of parasitoids using host location cues indirectly associated with the presence of their hosts.

Bark beetle boring dust or infested tree tissue, with or without bark beetles (*I. grandicollis*), were highly attractive to female *R. xylophagorum* (Paper IV). In contrast, isolated bark beetle larvae, or uninfested or artificially damaged tree tissue were not attractive. Qualitative and quantitative chemical differences were found in dynamic headspace samples, or steam distillates, from bark beetle damaged tree tissue (phloem and cambium of loblolly pine) versus non-damaged tree tissue (Paper IV).

Pupal chambers were most complex in chemical composition and emitted the greatest quantities of GC-EAD active compounds when analysing *R. tutela* (Paper VI). In contrast, only few compounds in minute amounts were detected in samples from isolated bark beetle larvae (*I. typographus*). Odour samples from pupal chambers with or without bark beetle larvae contained larger quantities of these compounds, implying that they originate from the tree tissue rather than the bark beetle larvae.

In efforts to identify the volatiles of potential use in oviposition site location, odour samples were collected within a few millimetres of susceptible hosts (close-range) (Paper VI). The GC-EAD active compounds detected in these samples were the same as the ones previously found in odour samples from whole logs (Papers II and V) or larger pieces of bark (Paper III and IV). Thus, no qualitative difference in volatile composition at oviposition sites could be detected as compared to previous analyses of long-range host location cues (host-habitat location cues). However, the thinner bark layer above pupal chambers most likely result in spatial variability in the compounds emitted, which would guide parasitoids in close-range host foraging. The possible consequences of the difference in bark thickness are discussed in Paper VI. The thinner bark above pupal chambers may either result in larger

release rates of volatiles since compounds produced in the phloem would pass through more easily. Alternatively, the thinner bark layer might result in lower release rates since a thinner phloem tissue could produce smaller quantities of compounds that attract parasitoids.

The GC-EAD active and attractive oxygenated monoterpenes, which arise from conifers containing susceptible bark beetle larvae, do not originate directly from the host organism but from the surrounding conifer tissue (phloem and cambium). Oxygenated monoterpenes probably arise through spontaneous oxidation of monoterpene precursors exposed to the air, metabolic processing of monoterpene hydrocarbons by invading insects and microorganisms, and other processes (Leufvén and Birgersson 1987; Leufvén et al. 1988; Hunt et al. 1989; Leufvén 1991; Smith et al. 1993).

Microorganisms seem to be important in this multitrophic interaction. The blue-stain fungi species found in association with *I. typographus* are different from the fungi associated with non-aggressive bark beetle species, indicating that pathogenic fungi are important for tree killing bark beetle species (Krokene and Solheim 1996). In contrast, the aggressive *D. frontalis* are able to kill trees (by mining that girdles the phloem) even without associated blue-stain fungi. Where there were less blue-stain fungi found in attacked trees, the *D. frontalis* outbreaks were most severe (Bridges et al. 1985). Thus, the interaction between bark beetles and blue-stain fungi may be different for different bark beetle/tree species. Laboratory infestations of axenic *I. grandicollis* (reared without microorganisms) still attract parasitoids, although "wild" beetle infestations (with associated microorganisms) are preferred over the former when given a choice of the two (B.T. Sullivan, unpublished data). Bridges and Moser (1986) found a positive correlation between the presence of blue-stain fungi and predatory mites attacking *D. frontalis*. The presence of bark beetle associated microorganisms thus seems to facilitate host foraging in parasitoids and predators of bark beetles.

This implies that microorganisms, directly or indirectly, enhance the production of volatile host location cues. The observed increased production of oxygenated monoterpenes as bark beetle larvae develop (E.M. Pettersson, unpublished data) coincides interestingly with a reported change in the tree phloem polyphenolic parenchyma cells (Franceschi et al. 2000). This physiological change has been suggested as an inducible host tree defence against bark beetles and microorganisms. Thus, the volatile host location cues used by these parasitoids seem to arise from some interaction between hosts, microorganisms, and the host tree.



## - CONCLUDING REMARKS -

This thesis shows that chemical cues are of vital importance to parasitic wasps attacking bark beetle larvae.

The odour perceptive abilities of the addressed parasitoids were investigated and synthetic parasitoid attractants were developed. A limited number of compounds, primarily oxygenated monoterpenes, are crucial in host foraging among these parasitoids (see "Main Results and Implications"). Interesting tasks for future research efforts include trying to unravel the chemical origin of these compounds, elucidating the role of enantiomers, and conducting a thorough screening of chemical and other cues that elicit oviposition.

The sophisticated interactions between parasitoids, bark beetles, associated microorganisms and host trees are truly fascinating, and a most interesting field of research. Increased knowledge about this complex chemical communication system is not only of utmost ecological and evolutionary interest, but is also of potential use in developing more efficient, environmentally safe, biological control techniques.

## Livsviktiga dofter - värdlokalisering hos parasitsteklar som angriper barkborrar

Vissa parasitsteklar (parasitoider) angriper och dödar barkborrelarver som ligger dolda under barken på barrträd. Steklarna påverkar således barkborrens populationstäthet. Syftet med det här forskningsprojektet var att utröna hur steklarna finner sin värd trots att det inte tycks finnas några synliga ledtrådar (se illustration s. 2 "Objectives").

I denna avhandling har ett fåtal nyckelsubstanser (dofter) identifierats som nödvändiga för värdlokaliseringen hos barkborre-parasitoider. Dessa substanser är främst oxygeneterade monoterpener och kommer inte i första hand från barkborrelarven utan från dess värdträd. De mikroorganismer som (i de flesta fall) introduceras i trädet genom ett barkborreangrepp tycks ha betydelse för produktionen av substanser som attraherar parasitoider.

De undersökta barkborreangripna barrträdsarterna, från Sverige och USA, avgav vissa kemiska substanser som attraherade parasitoider. Oavsett barkborre och barrträdsart, var dessa substanser i stort sett de samma. Efter kemisk analys av doftprover från naturligt material tillverkades syntetiska beten, med substanser som lockade parasitoidhonor i olika beteendetest.

Vissa av dessa parasitoidarter har tidigare använts, eller utvärderats, för biologisk kontroll av olika barkborrearter. Ökad kunskap om hur parasitoiderna finner sin värd gör det möjligt att vidareutveckla den typen av biologisk kontrollmetod. Det invecklade samspelet mellan parasitoider, barkborrar, mikroorganismer och värdträd är dessutom av stort ekologiskt och evolutionärt intresse.

## Parasitoider & Barkborrar

Parasitoider dödar per definition sin värd, vilket skiljer dem från parasiter som i allmänhet inte dödar sin värd. Vidare lever parasitoider främst på egen hand men kräver en värdorganism en begränsad period av livscykel, under sina tidiga levnadsstadier. Parasitoider kan tillhöra många olika insektsordningar men parasitsteklarna (Hymenoptera) är den överlägset största gruppen och utgör ca 20% av alla insektsarter, vilket motsvarar över 1 miljon arter. De parasitoider som avhandlas här är alla steklar och förekommer i stora delar av världen, deras vetenskapliga namn är:

Familj	Art	Äggläggning
Braconidae	<i>Coeloides bostrichorum</i>	genom barken
Pteromalidae	<i>Rhopalicus tutela</i>	genom barken
- - -	<i>Roptrocercus mirus</i>	i barkborrarnas gångsystem
- - -	<i>Roptrocercus xylophagorum</i>	i barkborrarnas gångsystem

Möjligen är den ringa storleken (3-10 mm) orsaken till att dessa steklar inte fått några svenska namn. Samtliga angriper flera olika arter av barkborrar som föryngrar sig i barrträd, vilket i Sverige främst innebär den åttatandade granbarkborren (*Ips typographus*, Coleoptera: Scolytidae) som här föryngrar sig i gran (*Picea abies*). I undersökningar av parasitoider från USA var värdbarkborrarna *Ips grandicollis* och *Dendroctonus frontalis* (vilka föryngrade sig i en amerikansk tall, *Pinus taeda*). Dessa tre barkborrearter har ekonomisk betydelse genom att de, när de förekommer i stor mängd, kan orsaka betydande värdeförluster i barrskogar.

## Värdlokalisering

Parasitoiderna lägger helst ägg på barkborrens sena larvstadium, som finns i puppkammare, dolda under granbarken. Den nykläckta parasitoidlarven äter sedan upp barkborrelarven. Det är emellertid inte helt enkelt för parasitoidhonan att hitta ett bra ställe för äggläggning. I en skog full av granar måste hon först hitta ett barkborreangripet träd som innehåller värddjur i rätt larvstadium, sedan återstår att lokalisera exakt rätt ställe att lägga sitt ägg på. Trots att det inte finns några synliga ledtrådar på barkytan lyckas både *R. tutela* och *C. bostrichorum* att lägga ägg på larven rakt igenom granbarken. De båda *Roptrocercus*-arterna går in i barkborregångarna och lokaliserar larven där.



Dofter är mycket betydelsefulla i många andra parasitoid-värd förhållanden. När det gäller värdlokalisering hos parasitoider på barkborrar är dock inte mycket känt sedan tidigare. Flera hypoteser har lagts fram om vad som vägleder parasitoiden till den dolda värden: vibrationer, värmestrålning (IR), och doft. Med undantag av doft, har övriga hypoteser förkastats.

## Doftforskningsmetodik & Resultat

Studier (elektronmikroskopi) av yttre och inre antennstrukturer visade en mångfald av kemoreceptorer Figure\* 4 (Fig. 1-7, Paper\* I). Detta indikerar att dofter har stor betydelse för parasitoiderna.

Doftprover insamlades från stockar och barkbitar med eller utan barkborrelarver. Olika artkombinationer av parasitoid-barkborre-barrträd undersöktes (Papers II-V). Ett antal doftsamlingsmetoder användes varav den mest använda beskrivs i Figure 1. Doftprover insamlades även från larver samt puppkammare med eller utan larver (Paper VI).

Insamlade doftprover innehåller en mångfald av substanser. Med hjälp av elektrofysiologi (GC-EAD, Figure 2) kan man urskilja de substanser som utlöser nervaktivitet i insektsantennen, vilka därmed är potentiella värdlokaliseringssubstanser (Table 2).

Doftproverna analyserades även kemiskt (GC-MS, Figure 3). De flesta GC-EAD aktiva substanserna identifierades som oxygenerade monoterpener, vilka indikerar skadade barrträd. Kontinuerlig analys av granstockar under ett barkborreangrepp, visade att avgivningen av vissa GC-EAD aktiva substanser ökar när barkborrelarver av "rätt" stadie börjar utvecklas under barken (ca 3 veckor efter barkborreangrepp), några av dessa substanser visas i Figure 5.

Ett antal syntetiska parasitoidbeten tillverkades, baserat på elektrofysiologisk och kemisk analys av insamlade doftprover. Dessa beten testades i beteendetest för att undersöka om de dofter som insekten kan känna är tilldragande, fränstötande, eller neutrala för den. Gående parasitoider undersöktes i "Y-tube walking bioassay" (Fig. 1, Paper II), medan vindtunnel (Figure 6) användes för flygande parasitoider. Vissa syntetiska beten var mycket attraktiva för parasitoidhonorna (Papers II, III och V). Minst tre substanser visade sig nödvändiga för att attrahera parasitoidhonor (Paper V).

Parasitoidhonor lockas starkast till stockar med barkborrelarver. Oangripna granstockar var inte attraherande, men i kombination med vissa syntetiska beten blev oangripna stockar lika attraherande som granstockar med barkborrelarver (Papers II och V).

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\* "Figure" hänvisar till figur i själva avhandlingen, medan "Fig." hänvisar till figur i en bifogad artikel ("Paper").

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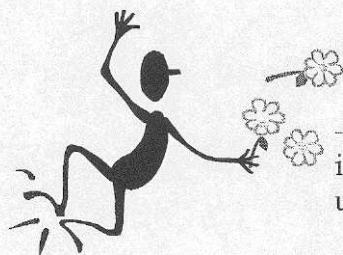
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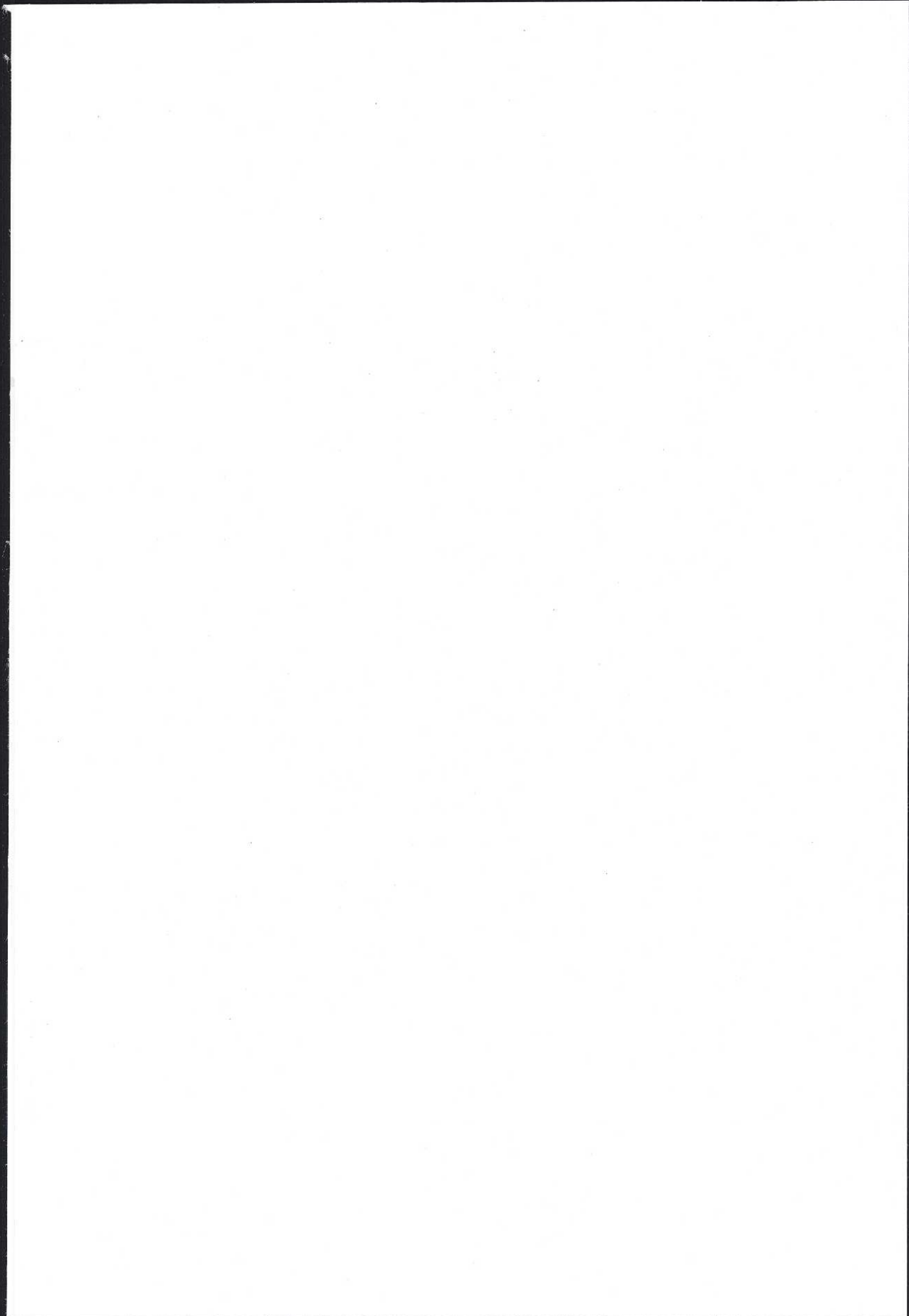
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