**DYNAMICS OF PHEROMONE PRODUCTION AND COMMUNICATION IN
THE MOUNTAIN PINE BEETLE, *Dendroctonus ponderosae* Hopkins AND THE
PINE ENGRAVER, *Ips pini* (Say) (COLEOPTERA: SCOLYTIDAE)**

by

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ABSTRACT

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins and the pine engraver, *Ips pini* (Say), often co-exist in the same host. Intra- and interspecific semiochemical communication occurs in both species. However, their complete semiochemical repertoire and precise dynamics of pheromone production have not been elucidated. Porapak-Q extracts of captured volatiles from beetles of both species aerated at different attack phases (freshly emerged, pioneer sex alone in the log and both sexes paired in new galleries), followed by gas chromatographic-electroantennographic detection (GC-EAD) and GC-mass spectroscopic analyses identified 17 compounds that excited the antennae of either or both species. Of these, there were seven compounds for *D. ponderosae* and nine for *I. pini*, that had not been assessed for behavioural activity. In field trapping experiments, 2-phenyl ethanol produced by both species inhibited the response of *D. ponderosae* to its aggregation pheromones. *exo*- and *endo*-Brevicomin produced by *D. ponderosae* significantly decreased the response of *I. pini* to its aggregation pheromone ipsdienol. Nonanal, a ubiquitous compound found in the volatiles of lodgepole pine, various nonhosts and in both beetle species deterred the response of *I. pini* to ipsdienol. The occurrence of *cis*-verbenol, *trans*-verbenol and verbenone in emergent *I. pini*, and verbenone and 2-phenyl ethanol in *D. ponderosae* suggests that these compounds may prevent aggregation and induce dispersal following emergence. GC analyses of 20 individuals of both sexes of each species at three attack phases revealed large differences in semiochemical content, but no relationship between size and production of aggregation pheromones. However, there was a significant correlation

between size and amount of antiaggregation pheromones in male *D. ponderosae*, suggesting that large males are best able to maintain their territories against members of both species. Large female *D. ponderosae* initiated longer galleries than small ones and secured males that produced more antiaggregation pheromones, implying better fitness. Large *I. pini* produced greater amounts of interspecific antiaggregants against *D. ponderosae* than small ones. Resource partitioning, population regulation and reproductive success in *I. pini* appears to depend largely on the rapid decline in the emission of ipsdienol after mate acquisition.

“Pheromones are the *effects* and not the causes in the evolution of mating systems”.

Kirkendall (1983)

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1. INTRODUCTION

1.1 The mountain pine beetle

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins, is the most lethal natural agent of lodgepole pine, *Pinus contorta* var. *latifolia* Engelman, in western North America (Furniss and Carolin 1977; Van Sickle 1989). Freshly eclosed adults leave their hosts between mid and late July in search of fresh host material (Reid 1962). Suitable hosts are located by visual and olfactory (kairomonal) cues (Billings et al. 1976; Raffa and Berryman 1982; Conn et al. 1983; Borden et al. 1983, 1987; Moeck and Simmons 1991). Females are the pioneer sex and initiate attack. As they begin to feed, they metabolise the monoterpene α -pinene to produce the aggregation pheromone *trans*-verbenol, which attracts both males and females to the attacked tree (Pitman et al. 1968, 1969; Billings 1976; Libbey et al. 1985). Males produce *exo*-brevicomin, which at low concentrations attracts more females (McKnight 1979; Borden et al. 1983; Conn et al. 1983; Libbey et al. 1985). These aggregation pheromones, synergised by host terpenes like α -pinene and myrcene mediate mass attack (Renwick and Vité 1970). During this process, the host is inoculated with pathogenic, mutualistic fungi that invade the phloem and sapwood increase the susceptibility of the tree by impeding translocation of water and nutrients to the crown (Whitney 1971). A sufficient number of beetles must aggregate to ensure enough fungal inoculum and mechanical damage to kill the tree (Wood 1982). A threshold density of 40 galleries per m² is required to overcome host resistance (Raffa and Berryman 1983). This ensures death of the tree and thereby, survival of both parent beetles and their progeny. Trees under attack may kill invading beetles by primary resinosis, pitching them out with a copious flow of resin from severed ducts in the phloem and sapwood (Callaham 1955). Trees with

relatively high vigour may supplement this with a secondary or hypersensitive response to attack (Reid et al. 1967; Berryman 1969, 1972). A series of metabolic processes occurs, whereby local autolysis of parenchyma cells, production of traumatic resin ducts and secondary resin with increased concentrations of monoterpenes and phenolic compounds result in a barrier that walls off the beetle and fungi within a lesion (Reid et al. 1967; Berryman 1969, 1972; Shrimpton 1973; Russell and Berryman 1976; Wong and Berryman 1977; Wright et al. 1979). Cellular necrosis and secretion of secondary resin then renders the phloem unsuitable for larval development (Raffa and Berryman 1982).

The phloem resource on the tree is limited and overcrowding results in intraspecific competition which is detrimental to the survival of brood. Once an optimum attack density (62 galleries per m²) is reached, aggregation is terminated by the production of high concentrations of the multifunctional pheromones *exo*-brevicomin and frontalin by males (Ryker and Rudinsky 1982; Ryker and Libbey 1982; Raffa and Berryman 1983; Borden et al. 1987) and verbenone by intestinal and gallery inhabiting microbes of both sexes (Leufvén et al. 1984; Hunt and Borden 1989, 1990). This signals to conspecifics the unavailability of fresh resource and switches the attack to adjacent trees in the vicinity (Rudinsky et al. 1974a; Geiszler and Gara 1978; Geiszler et al. 1980; Ryker and Libbey 1982; Ryker and Yandell 1983).

Males are monogynous. Following mating, eggs are laid along the walls of the vertical gallery (Raffa 1988). Larvae tunnel at right angles to the egg galleries while they feed and girdle the tree. Four larval instars are completed over the fall and the following spring. In the summer, pupation occurs, after which adults eclose (Reid 1962). Callow adults undergo maturation feeding for 3–4 weeks before emerging from the brood tree (Raffa 1988). In a mass attacked tree, the combined actions of the tunnelling adults, pathogenic fungi and feeding larvae disrupt translocation of water and nutrients to the crown, ultimately resulting in its death.

1.2 The pine engraver

The pine engraver, *Ips pini* (Say), is a secondary, weakly aggressive bark beetle, which breeds in the phloem of dead, moribund or stressed trees (Thomas 1961; Schenk and Benjamin 1969; Schmitz 1972). It commonly infests the upper and lower boles of lodgepole pine attacked by *D. ponderosae* (Hopping 1961; Furniss and Carolin 1977; Amman and Safranyik 1985; ¹Andrews 1987; Safranyik and Linton 1991). Occasionally, when populations build up in slash from windthrow or logging, *I. pini* can kill standing trees (Thomas 1961; Livingstone 1979). Adult beetles overwinter in logs or duff on the forest floor and emerge between May and September (Thomas 1961; Sartwell et al. 1971). Male pioneers use olfactory cues to locate hosts (Miller and Borden 1990). On initiating nuptial chambers, males produce the aggregation pheromone ipsdienol, which attracts both males and females to the site (Swaby and Rudinsky 1976; Birch et al. 1980; Lanier et al. 1980). Lanierone, another male produced compound, synergises aggregation in eastern populations (Teale et al. 1991; Miller et al. 1997). When females arrive, they prospect for males by assessing male sites as they walk on the bark (Reid and Stamps 1997). Once a female makes her choice, she excavates an egg gallery radiating from the nuptial chamber and lays eggs in niches along the gallery wall (Schmitz 1972). Males acquire an average of three females within three days of arriving on a host; however, the number of females may range from zero to eight (Thomas 1961; Schmitz 1972; Lissemore 1994; Reid and Stamps 1997). As soon as females are acquired, male attraction is rapidly terminated (Reid and Roitberg 1994). After mating, males remain in the gallery, defending it from intruders and actively clearing out frass that the females create as they tunnel (Schenk and Benjamin 1969; Reid and Roitberg 1994). The mechanism of termination of aggregation in *I. pini* has not been

¹ Andrews R.J. 1987. Engraver beetle attack in stands adjacent to logged areas in the western region of the Williams Lake TSA. Can. For. Serv., Pac. For. Cen. Pest Rep. (Unpublished).

elucidated and females are not known to produce any kind of pheromone (Borden et al. 1991, Lissemore 1997).

1.3 Interspecific interactions

I. pini frequently inhabits trees attacked by *D. ponderosae* resulting in exploitative competition for food and space between the two species (Hopping 1961; Amman and Safranyik 1985; Miller and Borden 1985; Poland and Borden 1998b). Tree killing bark beetles like *D. ponderosae* that rely on symbiotic fungi to predispose hosts to successful attack may have adapted to build galleries slowly so as to avoid outstripping the advance of their fungal symbionts. In contrast, like most secondary bark beetles that infest hosts with little or no capacity to resist attack, *I. pini* attacks rapidly and is a prolific breeder. It has a higher attack density than *D. ponderosae* and parents often re-emerge to establish second broods (Safranyik et al. 1996). The ability of *I. pini* to deplete resources quickly may give it a competitive edge when it attacks a tree already weakened by *D. ponderosae* (McCambridge and Knight 1972; Rankin and Borden 1991). *Dendroctonus ponderosae* larvae however, are larger than those of *I. pini* and may have an advantage in aggressive encounters (Rankin and Borden 1991). When *D. ponderosae* and *I. pini* attacked logs simultaneously, the number of emerging brood in both species decreased significantly and there was a reduction in the weight of emergent *I. pini* progeny (Rankin and Borden 1991). Therefore, mutual semiochemical inhibition would be an adaptive mechanism to avoid competition for the same resource (Borden 1975; Byers 1989) and minimise brood loss.

The complex pheromone systems of bark beetles influence interspecific resource partitioning (Flamm et al. 1989). Several investigations have documented semiochemical based communication among sympatric species of bark beetles. The presence of co-attacking species or their pheromones reduced the attack densities and reproductive success of *Dendroctonus*

rufipennis Kirby, *D. frontalis* Zimmermann, *D. ponderosae*, *D. brevicomis* LeConte, *Scolytus ventralis* LeConte, *I. pini* and *I. paraconfusus* Lanier (Miller and Keen 1960; Stark and Borden 1965; Ashraf and Berryman 1969; McCambridge and Knight 1972; Berryman 1973; Light and Birch 1979; Paine et al. 1981; Borden et al. 1991; Rankin and Borden 1991; Devlin and Borden 1995; Safranyik et al. 1996; Poland and Borden 1998a, 1998b). Studies by Hunt and Borden (1987) showed that ipsdienol, the aggregation pheromone of *I. pini* significantly reduced the attraction of *D. ponderosae* to myrcene, *exo*-brevicomin and *trans*-verbenol. In turn, the pheromones of *D. ponderosae* decreased the response of *I. pini* to traps baited with ipsdienol (Hunt and Borden 1987; Miller 1990). The complete semiochemical profiles of both *D. ponderosae* and *I. pini* and their full potential interactions have not been studied in detail.

1.4 Individual variation in pheromone production and its relationship with size

Pheromone production in bark beetles is essential for survival both at the level of the individual and the species. The striking complexity and variability of biological systems is often overlooked in studies using pooled extracts of volatiles from large groups of beetles for pheromone analyses (Borden et al. 1986). Few investigations have been conducted on individual variation in pheromone production in bark beetles (Schlyter and Birgersson 1989). Studies on *Ips typographus* (L.), *I. pini* and *D. ponderosae* revealed that most beetles produce low amounts of pheromone while a few produce high amounts (Birgersson et al. 1984; Borden et al. 1986; Hunt et al. 1986; Miller et al. 1989). The amount of *trans*-verbenol in female *D. ponderosae* exposed to α -pinene vapours varied from 0 to 2204 ng per beetle and there was a gradation of 0 to 2638 ng of ipsdienol in male *I. pini* fed on lodgepole pine (Borden 1986). Such wide-ranging individual variation suggests that there are intraspecific competitive interactions (Alcock 1982), wherein some individuals invest a large amount of metabolic energy in pheromone production,

while a majority establish galleries with relatively little fragrance and escape not only the metabolic cost of pheromone synthesis, but also the ecological and evolutionary costs of attracting predators and parasites (Borden 1986). These “sneakers” therefore, would survive by capitalising on the energy expended by the “pioneers” – an advantageous survival strategy.

One might hypothesise that these variations are related to the size of the animal, with the largest, most vigorous beetles producing the most pheromone and consequently, enjoying the greatest reproductive success. Size is an important factor in the fitness of bark beetles (Safranyik 1976; Sahota and Thompson 1979). Large female *D. ponderosae* have more fat reserves, disperse farther, survive extreme temperatures better and lay more eggs than small ones (Reid 1962; 1963; Atkins 1967; McGhehey 1971; Thompson and Bennett 1971; Amman 1972; Safranyik 1976; Amman and Cole 1983). Large male *I. pini* have better breeding opportunities and sire more offspring than small ones (Reid and Roitberg 1994; Robertson and Roitberg 1998). Although no size related assortative mating has been observed, female *I. pini* that actively prospect for males appear to prefer large males (Reid 1992; Reid and Stamps 1997). Large male *I. typographus* produced more sex pheromone than small ones (Anderbrant 1985), implying better fitness. Reid and Stamps (1997) hypothesise that information about male quality in *I. pini* is potentially available from male pheromones and may play a vital role in mate choice by female beetles. The possible relationships between size and pheromone production in *D. ponderosae* and *I. pini* are not known.

1.5 Objectives

Using *D. ponderosae* and *I. pini* as study species, my objectives were:

1. to determine the full potential semiochemical repertoire of both species by volatile capture at different phases of attack followed by coupled gas chromatographic-electroantennographic detection (GC-EAD) analysis (Gries 1992);
2. to test antennally-active compounds of unknown behavioural activity in the field and to determine their potential role in intra- and interspecific communication;
3. to elucidate the dynamics of semiochemical production by qualitative and quantitative analyses of volatiles at various phases of attack and
4. to study the relationship between size and semiochemical production and its possible fitness consequences.

2. MATERIALS AND METHODS

2.1 Collection of beetles and host material

Lodgepole pine trees naturally infested with *D. ponderosae* were felled near Princeton, B.C. between May and September 1998. Uninfested standing trees were felled and baited with ipsdienol to induce attack by *I. pini*. Infested logs were cut into bolts 0.5 m long and brought to the laboratory where the cut surfaces were sealed with paraffin. They were stored in a cold chamber at 4°C. Similarly, bolts from uninfested trees were sealed and stored outdoors until used.

Beetle infested bolts were placed in mesh-screen cages held at 24-27° C and sprayed with water every two days. Emergent beetles were collected in Petri dishes lined with moist filter paper and used immediately in experiments. *Dendroctonus ponderosae* was sexed using the dimorphism of the seventh abdominal tergite (Lyon 1958) and the sex of *I. pini* was determined using characters of the elytral declivity (Lanier and Cameron 1969).

2.2 Treatment of beetles

Beetles were classified into the following treatment groups: 1) Freshly emerged males and 2) females, 3) females (*D. ponderosae*) and males (*I. pini*) alone in log and 4) males and 5) females with mate(s) in new galleries. In treatment 3, the first attacking sexes of both species were freely allowed to attack a fresh lodgepole pine bolt in a screen-mesh cage. In treatments 4 and 5 for *D. ponderosae*, females were allowed to attack the bolt and after 24 h, males were supplied, allowing free mate choice to occur. Similarly, for *I. pini*, males were supplied with females after 24 h of boring. Excisions were made after 48 h of attack in treatment 3 for both species, after

96 h in treatments 4 and 5. Galleries were dissected using a pocket knife and beetles removed from the bark by gently prodding them with a plastic toothpick.

2.3 Collection and analysis of volatiles

2.3.1 Aerations of grouped beetles in glass tubes

Fifty beetles from each of the above attack phases were aerated as a pooled group in a modified version of Rudinsky's (1974) apparatus (Gries et al. 1992) until death, in Pyrex[®] tubes (1.2 cm OD, 18 cm long), by a water-aspirator drawn charcoal filtered air stream (airflow ca. 1.5 L per min). Volatiles were captured in a glass column (6 mm OD, 15 cm long) packed with 3 cm of Porapak-Q (50-80 mesh, Waters Associates, Inc., Milford, MA 01757) (Byrne et al. 1975).

Volatiles were eluted from the trap with 1 ml of distilled pentane using nitrogen gas at ca. 5 psi.

2.3.2 Gas chromatographic-electroantennographic detection (GC-EAD) and GC-mass spectroscopy (GC-MS)

Volatiles from tube aerations were subjected to GC-EAD analyses (Arn et al. 1975) past the antennae of males and females of both species. ²Figure 1 represents an example of a GC-EAD trace of the response by female *I. pini* and *D. ponderosae* antennae to the volatiles from male *I. pini*. A Varian 3400 gas chromatograph equipped with a fused silica column (DB-5, 30 m x 0.25 mm ID, J&W Scientific, Folsom, CA 95630) was employed. An indifferent electrode was placed in the head and a recording electrode in the antennal club of living beetles with the aid of a micromanipulator.

² GC-EAD analyses were performed by Ms. Regine Gries, Department of Biological Sciences, Simon Fraser University.

Figure 1. Antennal responses of female *I. pini* and female *D. ponderosae* to volatiles captured from an aeration of 50 male *I. pini* that were in a lodgepole pine bolt for 48 h.

Compounds that elicited an antennal response were identified by coupled GC-mass spectroscopy (MS) (Varian Saturn II). An ion trap equipped with the same column and a programmable injector was operated in the electron impact mode. The temperature programme was 1 min at 50°C and then 10°C/min to 200°C. The temperatures of the injection and detector ports were ca. 240°C. Calculation of retention indices (van den Dool and Kratz 1963), co-chromatography with authentic synthetic standards and spectral comparisons were done to identify antennally active compounds.

2.3.3 Quantification of antennally active compounds

Compounds that excited the antennae of beetles were quantified using 1 ng/μl of n-decanol as the internal standard on a Hewlett Packard 5890A gas chromatograph. Fused silica columns, DB-5 for *D. ponderosae* and DB-23 (30 m x 0.32 mm ID) for *I. pini* with settings as described in 2.3.2, were used to obtain separation of compounds. Identifications were confirmed by comparison of retention times and indices with those of authentic synthetic standards.

2.3.4 Individual beetle extractions

Twenty beetles of each species and sex from the five treatment groups were analysed individually for volatile content. In treatments 4 and 5 for the pine engraver, beetles were excised after 7 days after females were introduced. In addition, the length of each gallery was recorded. The length of each beetle from the anterior margin of pronotum to the posterior margin of the elytra (Robertson and Roitberg 1998) was measured to the nearest 0.01 mm, using a dissecting microscope equipped with an ocular micrometer. Beetles were also weighed (to the nearest 0.01 mg), on an electronic digital balance (Explorer Balances, Ohaus Corporation, NJ). They were then homogenised individually in 200 μl of hexane to which 5 ng/μl of n-decanol

was added as an internal standard. The extracts were filtered through glass wool and analysed by GC. All antennally active compounds were quantified. Since the internal standard was added to the solvent after the beetles were homogenised, the data were examined for any correlation between beetle size and the amount of internal standard. No relationship was observed, indicating that this methodology had no effect on the analysis.

2.3.5 Field infested lodgepole pine

A lodgepole pine tree was baited with *trans*-verbenol and *exo*-brevicomin on 5 August, 1998 to induce attack by *D. ponderosae*. The tree was felled on 9 August and brought to the laboratory. On 10 August, 10 paired males and females and 10 females that had not acquired males were excised from galleries. All beetles were treated as above and the antennally active compounds quantified.

2.3.6 Aerations of lodgepole pine

Aerations for host volatiles were done on four lodgepole pine bolts collected in May 1998, from three locations (Lytton, Sunday Summit and two trees from Whipsaw Creek, B.C.). The bolts were sawn into discs and aerated in 10 L plastic chambers. Air was drawn through the chamber for 45 h at 23°C with an airflow of ca. 2.5 L/min. Volatiles were captured in a glass trap (14 mm OD, 20 cm long) containing ca. 5 cm Porapak-Q (Byrne et al. 1975). The traps were eluted with 150 ml of distilled pentane and the extracts were concentrated to 4 ml by distillation of solvent through a 30 cm long Dufton column. The extracts were analysed on a Hewlett Packard 5890A gas chromatograph with settings as in 2.3.2. The amount of nonanal was quantified using synthetic nonanal as the external standard.

2.3.7 Field trapping experiments for behavioural activity

Compounds that elicited antennal responses and for which bioactivity was unknown, were tested for behavioural activity in the field between May and September 1998 in lodgepole pine forests near Princeton, B.C. Twelve-unit multiple-funnel traps (Lindgren 1983) were hung from ropes or poles ≥ 15 m apart in linear randomised complete blocks. Seven experiments were conducted for *D. ponderosae* and nine for *I. pini* (Table 1), with 11 replicates per experiment. Attractant-baited and unbaited control traps served as positive and negative controls, respectively. Each chemical was tested by itself and in combination with a proven attractant bait, myrcene, *trans*-verbenol and *exo*-brevicommin for *D. ponderosae* and ipsdienol for *I. pini*, to detect any potential enhancement or inhibition of attraction. For enantiomeric compounds, racemic blends were used. Captured insects from all experiments were frozen in plastic bags until sexed (Lyon 1958; Lanier and Cameron 1969) and counted.

2.4 Statistical analyses

2.4.1 Pheromone production dynamics

Data from the individual beetle extractions did not conform to the assumptions of normality and homoscedasticity demanded by parametric statistics. Therefore, Wilcoxon rank sums test and Kruskal-Wallis test for data with two and three treatments respectively, followed by non-parametric multiple comparisons for data with three treatments (Zar 1983, Day and Quinn 1989, SAS Inc. 1990), were used to determine differences between mean amounts of compounds across two or three attack phases for both species. In all cases $\alpha = 0.05$.

Table 1

Compounds tested for behavioural activity in the field against *D. ponderosae* and *I. pini*

Chemical	Source	Purity (%)	Release device	Target species ^a	Release rate (mg/24 h) ^b
nonanal	Aldrich Chemical Company Inc.	95	1.5 ml polypropylene microtube with 4 pores	PE	6.8
2-phenyl ethanol	Sigma Chemical Company	98	1.5 ml polypropylene microtube with 4 pores	MPB, PE	4.2
acetophenone	Aldrich Chemical Company Inc.	99	1.5 ml polypropylene microtube with 4 pores	MPB, PE	6.7
borneol	Sigma Chemical Company	88	20 ml low density polyethylene bottle with 16 pin pricks	MPB, PE	4.1
<i>p</i> -cymene	Aldrich Chemical Company Inc.	99	1.5 ml polypropylene microtube with 1 pore	MPB	6.9
<i>endo</i> -brevicommin	Phero Tech Inc.	93	250 µl polyethylene microtube with 8 pin pricks	PE	6.6
<i>exo</i> -brevicommin	Phero Tech Inc.	99	250 µl polyethylene microtube with 8 pin pricks	PE	3.1
(± <i>cis/trans</i>) limonene oxide	Aldrich Chemical Company Inc.	97	1.5 ml polypropylene microtube with 2 pores	MPB, PE	5.2
myrtenol	Aldrich Chemical Company Inc.	95	1.5 ml polypropylene microtube with 4 pores	MPB, PE	5.1
1-phenyl ethanol	Aldrich Chemical Company Inc.	98	1.5 ml polypropylene microtube with 4 pores	MPB, PE	5.9

^a MPB = mountain pine beetle, *D. ponderosae* ; PE = pine engraver, *I. pini*^b Release rates determined at 25°C in the laboratory

2.4.2 Field trapping experiments for behavioural activity

To satisfy the assumptions of normality and homoscedasticity, data were transformed by $\log_{10}(x+1)$ and analysed by ANOVA (GLM procedure) and the Ryan-Einot-Gabriel-Welsh Multiple Range (REGW) test (Zar 1983, Day and Quinn 1989, SAS Institute Inc. 1990). In all cases $\alpha = 0.05$.

2.4.3 Individual variation in pheromone production and its relationship with size

The quantitative profiles of pheromones in 20 individual beetles per sample were plotted for males and females in all attack phases for both species in the laboratory. The Kolmogorov-Smirnov goodness of fit test for continuous distributions (Zar 1983) was used to analyse the variation in the amount of compounds present in individual beetles ($\alpha = 0.05$). Since the data did not satisfy the assumptions for parametric statistics, Spearman rank correlation coefficients ($\alpha = 0.10$) between size and pheromone production were calculated using both length and weight separately as indices of size. Spearman correlation coefficients were also calculated to determine relationships between amounts of different pheromone components within the same sex and between gallery length and pheromone production, for both species. For *D. ponderosae* from the field attacked tree, correlation coefficients of pheromone amounts between males and females in the same gallery and between female size and male pheromone production were determined.

3. RESULTS

3.1 Production dynamics

3.1.1 Aerations of grouped beetles in glass tubes

3.1.1.1 *D. ponderosae*

Twelve compounds found in the volatiles of *D. ponderosae* elicited an antennal response from either or both species (Table 2, Figure 2). *exo*-Brevicommin and *endo*-brevicommin were detected both in freshly emerged and paired males but not in females. *trans*-Verbenol was seen in large amounts in females at all three phases of attack. Frontalin was detected only in paired males. Verbenone was present in both sexes at all phases of attack, with large amounts in females that were in the log alone and lower amounts in freshly emerged and paired females. 2-Phenyl ethanol and nonanal were detected in both sexes at all phases but were present in larger amounts in freshly emerged beetles than in the other two phases. *p*-Cymene was detected only in freshly emerged beetles. Acetophenone was present in both sexes at all phases and more limonene oxide was present in freshly emerged beetles than in the other two phases. Borneol was detected only in females at all three phases.

3.1.1.2 *I. pini*

Eleven compounds found in the volatiles of *I. pini* excited the antennae of either or both species (Table 2, Figure 3). Ipsdienol, seen in trace amounts in freshly emerged males rose dramatically in males that had been in the log for 48 h and then declined to its original amount once they acquired mates. Trace amounts were detected in paired females. Lanierone was present only in males and did not differ in amount at any of the three phases. *cis*-Verbenol, *trans*-verbenol and verbenone were released in large amounts in freshly emerged beetles of both sexes. Nonanal was detected both in beetles that were in the log alone and in those that had acquired mates.

Table 2 Compounds detected in the volatiles of *D. ponderosae* and *I. pini* aerated in tubes and their antennal response.

Source species and compound	Sex in which compound was detected					
	<i>D. ponderosae</i>			<i>I. pini</i>		
	males	females		males	females	
Both species						
<i>cis</i> -verbenol	+	+		+	+	+
<i>trans</i> -verbenol		+		+		+
verbenone	+	+		+	+	+
nonanal	+	+		+	+	+
2-phenyl ethanol	+	+		+	+	+
acetophenone	+	+		+	+	+
borneol	+	+		+	+	+
<i>D. ponderosae</i>						
<i>exo</i> -brevicommin	+					+
<i>endo</i> -brevicommin	+					+
frontalin	+					+
<i>p</i> -cymene	+					+
<i>cis/trans</i> -limonene oxide	+	+				+
<i>I. pini</i>						
ipsdienol				+		+
lanierone				+		+
1-phenyl ethanol				+		+
myrtenol				+		+

Figure 2. Amounts of antennally-active compounds per beetle as determined by gas chromatographic analyses of pooled volatiles from 50 male and 50 female *D. ponderosae* aerated at two and three attack phases, respectively. Note variable scales for amounts per beetle. NA = Not Applicable.

Figure 3. Amounts of antennally-active compounds per beetle as determined by gas chromatographic analyses of pooled volatiles from 50 male and 50 female *I. pini* aerated at three and two attack phases, respectively. Note variable scales for amounts per beetle. NA = Not Applicable.

2-Phenyl ethanol produced both by both sexes, increased in amount in males that were in the log for 48 h. Acetophenone and 1-phenyl ethanol were produced by freshly emerged females and also detected in paired males in relatively high amounts. Borneol was present in high amounts in the volatiles of paired beetles, while myrtenol was detected only in freshly emerged beetles of both sexes.

3.1.2 Individual beetle extractions

3.1.2.1 *D. ponderosae*

Results from the individual beetle extractions (Figure 4) reflected those for the group aerations for *exo*-brevicomin, frontalin, nonanal, *p*-cymene and acetophenone. A small amount of *endo*-brevicomin was present in freshly emerged males, but unlike the group aerations, none was present in paired males. *trans*-Verbenol was absent in emerged females, significantly reduced in paired females and present in 40% of paired males. In contrast to the pooled aerations, verbenone was not found in emerged beetles of either sex, but consistent with the group aerations, was found in significantly high amounts in paired males and in females in the log alone. 2-Phenyl ethanol was found in large amounts in paired males, but unlike the pooled aerations, was absent in emerged beetles. Only one emerged beetle contained limonene oxide and paired males contained low but significant amounts of the *trans*-isomer. Borneol was not detected in emerged beetles, but was present in four paired males.

3.1.2.2 *D. ponderosae* excised from a tree attacked in the field

Mean amounts of compounds from *D. ponderosae* that were excised from the field-attacked tree are shown in Table 3. Paired females had significantly more *trans*-verbenol and verbenone than females without mates. Females had trace amounts of *exo*-brevicomin, *endo*-brevicomin and

Figure 4. Mean amounts of antennally-active compounds as determined by gas chromatographic analyses of individual extracts from 20 male and 20 female *D. ponderosae* at each of two or three attack phases, respectively. Note variable scales for amounts per beetle. Bars with the same letter are not significantly different, nonparametric multiple comparisons, $P < 0.05$. Numbers in or beside bars indicate number of beetles containing detectable amounts of compound. NA = Not Applicable.

□ EMERGED
 ■ IN LOG ALONE
 ■ IN LOG WITH MATES

Table 3 Mean amount ($\pm$ SE) of pheromone and beetle associated compounds in *D. ponderosae* from the field attacked tree. For females, data within a row followed by the same letter are not significantly different, Wilcoxon's rank sum test, $P < 0.05$. Significant values are in bold.

Compound	Amount per beetle in ng (mean $\pm$ SE)		
	paired males	paired females	single females
<i>cis</i> -verbenol	50.0 $\pm$ 13.4	46.4 $\pm$ 13.6 a	40.2 $\pm$ 18.0 a
<i>trans</i>-verbenol	66.4 $\pm$ 24.8	458.0 $\pm$ 77.8 b	279.4 $\pm$ 100.2 a
<i>exo</i> -brevicommin	61.4 $\pm$ 14	8.8 $\pm$ 3.8 a	23.4 $\pm$ 7.8 a
<i>endo</i> -brevicommin	7.4 $\pm$ 2.2	5.6 $\pm$ 2.8 a	12.6 $\pm$ 4.6 a
frontalin	150.0 $\pm$ 26.6	2.4 $\pm$ 1.0 a	23.8 $\pm$ 18.4 a
verbenone	66.0 $\pm$ 11.4	51.4 $\pm$ 7.2 b	24.6 $\pm$ 3.4 a
2-phenyl ethanol	26.8 $\pm$ 5.6	25.4 $\pm$ 6.2 a	31.0 $\pm$ 3.8 a
nonanal	11.0 $\pm$ 1.0	7.0 $\pm$ 0.8 a	10.2 $\pm$ 0.8 a
<i>p</i> -cymene	93.0 $\pm$ 18.4	68.0 $\pm$ 17.8 a	114.8 $\pm$ 28.8 a
acetophenone	20.6 $\pm$ 4.2	33.8 $\pm$ 5.6 a	35.4 $\pm$ 4.8 a
<i>cis</i> -limonene oxide	3.2 $\pm$ 1.2	5.8 $\pm$ 2.0 a	12.2 $\pm$ 3.4 a
<i>trans</i> -limonene oxide	5.6 $\pm$ 1.8	7.8 $\pm$ 2.6 a	17.2 $\pm$ 4.6 a
borneol	11.4 $\pm$ 1.6	12.6 $\pm$ 1.4 a	16.2 $\pm$ 1.6 a

frontalin. There was no significant difference in the amounts of any of the other compounds between paired females and those without mates. Comparisons between males and females were not performed.

3.1.2.3 *I. pini*

Quantitative analyses of compounds detected in individual *I. pini* (Figure 5) were similar to the group aerations only for ipsdienol which was produced in significant amounts only by males after they were in the log for 48 h. Lanierone was detected in freshly emerged males but there was a significant drop in the amount detected in males in the other two phases. Both *cis*- and *trans*-verbenol were seen in freshly emerged females, while *cis*-verbenol alone was detected in paired males. Significantly more verbenone was detected in males that were in the log alone and in paired females than in the other phases. Nonanal was present in both sexes at all phases. 2-Phenyl ethanol was seen in significant amounts in both sexes of emerged beetles and particularly in males that were in the log alone. Freshly emerged beetles of both sexes contained acetophenone and borneol. 1-Phenyl ethanol and myrtenol were not quantified because they occurred in trace amounts.

3.2 Aerations of lodgepole pine

GC-MS analyses revealed the presence of nonanal in all four samples from uninfested lodgepole pine (Table 4). Between 0.4 and 1.8 ng/h/g tissue was captured in the aerations.

3.3 Field trapping experiments for determination of behavioural activity

Of the seven compounds tested against *D. ponderosae* (Figure 6), only 2-phenyl ethanol was behaviourally active. It significantly reduced the attraction of both sexes to the aggregation

Figure 5. Mean amounts of antennally-active compounds as determined by gas chromatographic analyses of individual extracts from 20 male and 20 female *I. pini* at each of three or two attack phases, respectively. Note variable scales for amounts per beetle. Bars with the same letter are not significantly different, nonparametric multiple comparisons, $P < 0.05$. Numbers in or beside bars indicate number of beetles containing detectable amounts of compound. NA = Not Applicable.

Table 4 Amount of nonanal detected in volatiles captured from discs cut from the boles of freshly felled lodgepole pine from three locations in Southern B.C.

Source	Amount captured by aeration (ng/h/g tissue)	Percentage of total volatiles
Lytton	1.736	0.050
Sunday Summit	0.473	0.059
Whipsaw Creek	0.778	0.032
Whipsaw Creek	1.756	0.051

Figure 6. Effect of previously untested antennally active compounds on the numbers of *D. ponderosae* captured in multiple funnel traps near Princeton B.C. between May and August 1998. Note variable scale for numbers of beetles captured. Bars (n = number of replicates) with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

pheromone (males: $F = 5.3$, $df = 13,30$, $P < 0.0001$, females: $F=8.78$, $df = 12, 27$, $P < 0.0001$). Of the nine compounds tested against *I. pini*, nonanal (males: $F = 4.1$, $df = 13,30$, $P < 0.0007$, females: $F = 6.28$, $df = 13,30$, $P < 0.0001$), *exo*-brevicomin (males: $F = 42.24$, $df = 13,30$, $P < 0.0001$, females: $F=96.44$, $df = 13,30$, $P < 0.0001$) and *endo*-brevicomin (males: $F = 9.63$, $df = 13,30$, $P < 0.0001$, females: $F = 23.37$, $df = 13,30$, $P < 0.0001$) inhibited the response *I. pini* to ipsdienol (Figure 7). Despite the fact that the presence of some untested compounds were species specific in production, i.e. *p*-cymene and limonene oxide for *D. ponderosae* and 1-phenyl ethanol and myrtenol for *I. pini* (Table 2), none showed any behavioural activity.

3.4 Individual variation in pheromone production and its relationship with size

3.4.1 *D. ponderosae*

Except for verbenone and nonanal in female *D. ponderosae*, that were in the log alone, and frontalin, verbenone and 2-phenyl ethanol in paired males, individual variation in the amounts of behaviourally active compounds was highly significant (Figures 8-11), indicating a strong trend for most beetles to produce very little material and a few to produce large quantities. There was no significant correlation between size and pheromone production in freshly emerged females or in females that were in the log alone (Figure 8). In paired females (Figure 9), there was a significant positive relationship between beetle weight and the amount of verbenone, as well as between beetle length and weight and 2-phenyl ethanol production. In freshly emerged males (Figure 10), there was no relationship between size and pheromone production. In paired males however, (Figure 11), there were significant positive correlations between size and the amount of *exo*-brevicomin, frontalin, verbenone and 2-phenyl ethanol.

For male *D. ponderosae* that were excised from the field attacked tree, there were significant positive relationships between female gallery lengths and amounts of

Figure 7. Effect of previously untested antennally active compounds on the numbers of *I. pini* captured in multiple funnel traps near Princeton B.C. between May and August 1998. Note variable scale for numbers of beetles captured. Bars (n = number of replicates) with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

Figure 8. Ranked variations in production of behaviourally active volatiles (left hand column) by 20 freshly emerged female *D. ponderosae* and 20 females alone in a log compared to the same amounts plotted in relation to length (middle column) and weight (right hand column) of the individually extracted beetles. For ranked variations, significant values for the Kolmogorov-Smirnov *D* statistic (NS = Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) indicate skewed departure from a regular continuous progression. For associations between length and weight with volatile content, Spearman rank correlation coefficients (*r*) with $P < 0.10$ indicate a significant relationship.

FRESHLY EMERGED

IN LOG ALONE

Figure 9. Ranked variations in production of behaviourally active volatiles (left hand column) by 20 female *D. ponderosae* paired with males compared to the same amounts plotted in relation to length (middle column) and weight (right hand column) of the individually extracted beetles. For ranked variations, significant values for the Kolmogorov-Smirnov *D* statistic (NS = Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) indicate skewed departure from a regular continuous progression. For associations between length and weight with volatile content, Spearman rank correlation coefficients (*r*) with $P < 0.10$ indicate a significant relationship.

Figure 10. Ranked variations in production of behaviourally active volatiles (left hand column) by 20 freshly emerged male *D. ponderosae* compared to the same amounts plotted in relation to length (middle column) and weight (right hand column) of the individually extracted beetles. For ranked variations, significant values for the Kolmogorov-Smirnov D statistic (NS = Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) indicate skewed departure from a regular continuous progression. For associations between length and weight with volatile content, Spearman rank correlation coefficients (r) with $P < 0.10$ indicate a significant relationship.

Figure 11. Ranked variations in production of behaviourally active volatiles (left hand column) by 20 male *D. ponderosae* paired in galleries with females compared to the same amounts plotted in relation to length (middle column) and weight (right hand column) of the individually extracted beetles. For ranked variations, significant values for the Kolmogorov-Smirnov *D* statistic (NS = Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) indicate skewed departure from a regular continuous progression. For associations between length and weight with volatile content, Spearman rank correlation coefficients (*r*) with $P < 0.10$ indicate a significant relationship.

exo-brevicomin, frontalin, verbenone and nonanal (Table 6). In females, all semiochemicals were negatively associated with gallery length but were not significantly correlated. Correlation analyses between different pheromone components in *D. ponderosae* revealed that beetles with high amounts of one pheromone component tended to have high amounts of other components within the same attack phase (Tables 5, 7). These results were consistent for both sexes in beetles which had attacked logs in the laboratory as well as in the field.

There was a high positive correlation between gallery length and size of females which had not acquired males in the field attacked tree (Figure 12). Significant positive relationships existed between female length and weight and the amount of nonanal and frontalin detected in the males with which they were paired (Table 8). Similarly, significant relationships occurred between female length and male verbenone content as well as between female weight and male 2-phenyl ethanol content. Finally, the amount of semiochemical in paired females was negatively associated (significantly in six instances) with the semiochemical content of their male mates (Table 9).

3.4.2 *I. pini*

Except for the production of lanierone in freshly emerged males, 2-phenyl ethanol in freshly emerged females and nonanal in paired female *I. pini*, there was significant variation in the amount of all other compounds at all attack phases (Figures 13-17), indicating that as in *D. ponderosae*, most *I. pini* produce very low amounts of semiochemicals and a few beetles produce large quantities.

There was no relationship between size and semiochemical production in freshly emerged males (Figure 13). In males that were in the log alone (Figure 14), there were significant positive relationships between beetle length and the amount of verbenone and

Table 5 Ranked relationships between amounts of semiochemicals produced by individual *D. ponderosae* within the same sex and attack phase. Spearman correlation coefficients with $P < 0.10$ indicate a significant relationship.

Sex	Phase of attack	Compounds compared	Spearman correlation coefficient	P
Females	in log alone	<i>trans</i> -verbenol, verbenone	0.826	0.0001
		<i>trans</i> -verbenol, nonanal	0.644	0.0022
		verbenone, nonanal	0.606	0.0046
		<i>trans</i> -verbenol, 2-phenyl ethanol	0.447	0.0481
		verbenone, 2-phenyl ethanol	0.426	0.0614
		2-phenyl ethanol, nonanal	0.420	0.0656
Males	paired	verbenone, 2-phenyl ethanol	0.648	0.002
		<i>trans</i> -verbenol, 2-phenyl ethanol	0.647	0.002
		<i>trans</i> -verbenol, verbenone	0.584	0.0069
		2-phenyl ethanol, nonanal	0.551	0.0117
	freshly emerged	<i>exo</i> -brevicommin, <i>endo</i> -brevicommin	0.704	0.0005
	paired	frontalin, 2-phenyl ethanol	0.863	0.0001
		verbenone, 2-phenyl ethanol	0.746	0.0002
		<i>exo</i> -brevicommin, frontalin	0.713	0.0004
		<i>exo</i> -brevicommin, verbenone	0.663	0.0014
		<i>exo</i> -brevicommin, <i>trans</i> -verbenol	0.642	0.0023
		verbenone, frontalin	0.591	0.0061
		verbenone, <i>trans</i> -verbenol	0.509	0.0218
		frontalin, nonanal	0.480	0.0323
		2-phenyl ethanol, nonanal	0.438	0.0537

Table 6 Ranked Spearman correlation coefficients for relationships between gallery length and semiochemical content in paired *D. ponderosae* (n = 10 beetles of each sex) excised from a field attacked lodgepole pine five days after semiochemical baiting on 5 August 1998 to induce attack. Significant values ($P < 0.10$) are in bold.

Sex	Compound	Spearman correlation coefficient	P
male	<i>exo</i> -brevicomin	0.912	0.0002
	frontalin	0.784	0.0072
	verbenone	0.711	0.0211
	nonanal	0.62	0.0558
	trans-verbenol	0.457	0.184
	<i>cis</i> - verbenol	0.433	0.2114
	<i>endo</i> -brevicomin	0.103	0.776
	2-phenyl ethanol	-0.407	0.2427
female	<i>endo</i> -brevicomin	-0.105	0.7734
	2-phenyl ethanol	-0.109	0.7635
	<i>exo</i> -brevicomin	-0.143	0.6942
	frontalin	-0.216	0.5494
	nonanal	-0.334	0.345
	verbenone	-0.377	0.283
	<i>trans</i> -verbenol	-0.468	0.1725
	<i>cis</i> -verbenol	-0.485	0.1556

Table 7 Relationships between amounts of semiochemicals produced by male and female *D. ponderosae* excised from a field attacked lodgepole pine five days after semiochemical baiting to induce attack. Spearman correlation coefficients in bold with $P < 0.10$ indicate a significant relationship.

Sex	Compound	frontalin	exo-brevicomin	endo-brevicomin	cis-verbenol	trans-verbenol	verbenone	2-phenyl ethanol	nonanal
male	frontalin	r = 0.830 P = 0.0029	r = -0.119 P = 0.743	r = 0.316 P = 0.3736	r = 0.219 P = 0.5436	r = 0.612 P = 0.0600	r = -0.115 P = 0.7514		r = 0.479 P = 0.1615
	exo-brevicomin		r = 0.206 P = 0.5673	r = 0.3830 P = 0.2747	r = 0.353 P = 0.3177	r = 0.650 P = 0.0425	r = -0.212 P = 0.5563		r = 0.467 P = 0.1739
	endo-brevicomin			r = 0.044 P = 0.9041	r = 0.097 P = 0.7893	r = -0.394 P = 0.2600	r = -0.313 P = 0.3791		r = -0.469 P = 0.1715
	cis-verbenol				r = 0.960 P = 0.0001	r = 0.657 P = 0.0392	r = -0.675 P = 0.0323		r = 0.195 P = 0.5902
	trans-verbenol					r = 0.590 P = 0.0728	r = -0.790 P = 0.0065		r = 0.152 P = 0.6751
	verbenone						r = -0.297 P = 0.4047		r = 0.733 P = 0.0158
	2-phenyl ethanol								r = 0.006 P = 0.9867
	nonanal								
	female	frontalin	r = 0.656 P = 0.040	r = 0.848 P = 0.0019	r = 0.760 P = 0.0107	r = 0.485 P = 0.1556	r = 0.143 P = 0.6927	r = 0.048 P = 0.8957	
exo-brevicomin			r = 0.851 P = 0.0018	r = 0.681 P = 0.0302	r = 0.369 P = 0.2947	r = -0.032 P = 0.9294	r = -0.252 P = 0.4822		r = 0.705 P = 0.0229
endo-brevicomin				r = 0.651 P = 0.0416	r = 0.500 P = 0.1415	r = -0.052 P = 0.8861	r = 0.201 P = 0.5770		r = 0.768 P = 0.0095
cis-verbenol					r = 0.523 P = 0.1210	r = 0.395 P = 0.2584	r = -0.061 P = 0.8675		r = 0.638 P = 0.0470
trans-verbenol						r = 0.236 P = 0.5109	r = 0.200 P = 0.5796		r = 0.248 P = 0.4888
verbenone							r = -0.212 P = 0.5563		r = 0.042 P = 0.9074
2-phenyl ethanol									r = 0.127 P = 0.7261
nonanal									

Figure 12. Relationships between gallery length and size of female *D. ponderosae* that had not acquired males five days after a lodgepole pine was baited with semiochemicals to induce attack. Spearman rank correlation coefficients with $P < 0.10$ indicate a significant relationship.

Table 8 Relationship between female size and male semiochemical production in *D. ponderosae* excised from a field attacked lodgepole pine five days after semiochemical baiting to induce attack. Spearman correlation coefficients in bold with $P < 0.10$ indicate a significant relationship.

Compound in males	Female length (mm)		Female weight (mg)	
	Spearman correlation coefficient	P	Spearman correlation coefficient	P
nonanal	0.711	0.0213	0.612	0.0600
frontalin	0.617	0.0574	0.588	0.0739
verbenone	0.542	0.1054	0.406	0.2443
2-phenyl ethanol	0.461	0.1797	0.588	0.0739
<i>exo</i> -brevicomin	0.449	0.1933	0.418	0.2291

Table 9 Ranked relationships between amounts of semiochemical components between male and female *D. ponderosae* in a field attacked tree five days after a lodgepole pine was semiochemical baited to induce attack. Spearman rank correlation coefficients in bold with $P < 0.10$ indicate a significant relationship.

Compound in females	Compound in males	Spearman correlation coefficient	<i>P</i>
<i>cis</i> -verbenol	frontalin	-0.681	0.0302
<i>trans</i> -verbenol	nonanal	-0.648	0.0425
verbenone	frontalin	-0.576	0.0816
2-phenyl ethanol	<i>trans</i> -verbenol	0.571	0.0844
<i>trans</i> -verbenol	<i>exo</i> -brevicommin	-0.564	0.0897
<i>cis</i> -verbenol	<i>exo</i> -brevicommin	-0.553	0.0972
<i>trans</i> -verbenol	frontalin	-0.539	0.1076
<i>trans</i> -verbenol	verbenone	-0.527	0.1173
<i>cis</i> -verbenol	verbenone	-0.505	0.1369
verbenone	<i>exo</i> -brevicommin	-0.430	0.2145
verbenone	verbenone	-0.430	0.2145

Figure 13. Ranked variations in production of behaviourally active volatiles (left hand column) by 20 freshly emerged male *I. pini* compared to the same amounts plotted in relation to length (middle column) and weight (right hand column) of the individually extracted beetles. For ranked variations, significant values for the Kolmogorov-Smirnov *D* statistic (NS = Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) indicate skewed departure from a regular continuous progression. For associations between length and weight with volatile content, Spearman rank correlation coefficients (*r*) with $P < 0.10$ indicate a significant relationship.

Figure 14. Ranked variations in production of behaviourally active volatiles (left hand column) by 20 male *I. pini* that were in a log alone for 48 h compared to the same amounts plotted in relation to length (middle column) and weight (right hand column) of the individually extracted beetles. For ranked variations, significant values for the Kolmogorov-Smirnov *D* statistic (NS = Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) indicate skewed departure from a regular continuous progression. For associations between length and weight with volatile content, Spearman rank correlation coefficients (*r*) with $P < 0.10$ indicate a significant relationship.

Figure 15. Ranked variations in production of behaviourally active volatiles (left hand column) by 20 male *I. pini* that were with ≥ 1 females in galleries compared to the same amounts plotted in relation to length (middle column) and weight (right hand column) of the individually extracted beetles. For ranked variations, significant values for the Kolmogorov-Smirnov *D* statistic (NS = Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) indicate skewed departure from a regular continuous progression. For associations between length and weight with volatile content, Spearman rank correlation coefficients (*r*) with $P < 0.10$ indicate a significant relationship.

Figure 16. Ranked variations in production of behaviourally active volatiles (left hand column) by 20 freshly emerged female *I. pini* compared to the same amounts plotted in relation to length (middle column) and weight (right hand column) of the individually extracted beetles. For ranked variations, significant values for the Kolmogorov-Smirnov *D* statistic (NS = Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) indicate skewed departure from a regular continuous progression. For associations between length and weight with volatile content, Spearman rank correlation coefficients (*r*) with $P < 0.10$ indicate a significant relationship.

VARIATION IN INDIVIDUAL BEETLES

LENGTH (mm)

WEIGHT (mg)

Figure 17. Ranked variations in production of behaviourally active volatiles (left hand column) by 20 female *I. pini* that were in galleries with males compared to the same amounts plotted in relation to length (middle column) and weight (right hand column) of the individually extracted beetles. For ranked variations, significant values for the Kolmogorov-Smirnov *D* statistic (NS = Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) indicate skewed departure from a regular continuous progression. For associations between length and weight with volatile content, Spearman rank correlation coefficients (*r*) with $P < 0.10$ indicate a significant relationship.

2-phenyl ethanol. In paired males (Figure 15), there was a significant negative correlation between beetle length and the amount of nonanal. The amount of *trans*-verbenol was positively correlated with both length and weight of freshly emerged females (Figure 16) and with length alone in females with males (Figure 17). The amount of *trans*-verbenol in females had a significant positive relationship with gallery length ($r = 0.481$, $P = 0.0233$). Except for nonanal and verbenone in freshly emerged males, for which a negative relationship was seen, the amounts of all other compounds were positively correlated with each other (Table 10).

Table 10 Relationships between amounts of semiochemicals in *I. pini* within the same sex and attack phase. Spearman rank correlation coefficients with $P < 0.10$ indicate a significant relationship.

Sex	Phase of attack	Compounds compared	Spearman correlation coefficient	P
Male	freshly emerged	nonanal, verbenone	-0.440	0.0520
	in log alone	ipsdienol, verbenone	0.523	0.0179
		lanierone, verbenone	0.381	0.0973
	paired in log	<i>cis</i> -verbenol, lanierone	0.580	0.0117
Female	freshly emerged	nonanal, <i>cis</i> -verbenol	0.451	0.0459
		nonanal, <i>trans</i> -verbenol	0.55	0.0119
		<i>cis</i> -verbenol, <i>trans</i> -verbenol	0.48	0.0323
		<i>trans</i> -verbenol, verbenone	0.485	0.0303
	paired females	<i>cis</i> -verbenol, verbenone	0.518	0.0136

4. DISCUSSION

4.1 Pheromone production dynamics

4.1.1 *D. ponderosae*

Data from the tube aerations and individual extractions were not entirely consistent (Figures 2, 4). The lack of *trans*-verbenol and verbenone in individual extractions of emergent beetles is consistent with previous studies (Pitman and Vité 1969; Hughes 1973a,1973b; Gries et al. 1990b). Detection of *trans*-verbenol from the volatiles of pooled emerged beetles in aeration tubes was possibly due to the oxidation of α -pinene in the guts of dying beetles either spontaneously or by intestinal microbes (Hunt et al. 1989; Hunt and Borden 1990). Under normal conditions, emerged beetles may avoid being attractive by metabolising *trans*-verbenol into verbenene, *p*-mentha-1,5,8-triene or cymene (Gries et al.1990b). *trans*-Verbenol was detected in high amounts in both aerations and extractions of fed females, which is consistent with the findings of Pierce et al. (1987).

exo- and *endo*-Brevicomin were detected in male *D. ponderosae* at both phases of attack while frontalin was present only in males paired with females. Trace amounts of brevicomin and frontalin were seen in females from the field-attacked tree (Table 3). Pitman et al. (1969) detected trace amounts of brevicomin in emergent and fed females, but Pierce et al. (1987) did not detect either brevicomin or frontalin in female volatiles. Under natural conditions, females may use trace amounts of frontalin and brevicomin to synergise the aggregation potential of *trans*-verbenol.

Verbenone has antiaggregative properties for a number of scolytid beetles (Shore et al. 1992; Borden 1997) and was detected in the captured volatiles of emerged and paired beetles of both sexes. It was previously identified in the hindguts of emergent and feeding females and in the volatiles of paired beetles (Pitman et al. 1969; Rudinsky et al. 1974a). The presence of an

antiaggregant in the volatiles of emerged beetles indicates its possible function in inducing dispersal. *trans*-Verbenol, an antiaggregant for *D. brevicomis*, was detected at early stages of colonisation, indicating a function in attack density regulation (Byers and Wood 1980; Byers 1983). The presence of verbenone in the volatiles of female *D. ponderosae* that were alone in a log suggests that it may function in spatial regulation of attack and that its influence can be overpowered by the aggregation pheromones *trans*-verbenol and *exo*-brevicomin at the onset of attack. The occurrence of large amounts of verbenone at the beginning of colonisation and its simultaneous decline with the amounts of attractive compounds in *D. brevicomis* led to the speculation that reduction in the amount of aggregation pheromones terminated aggregation, while verbenone served as a short range density regulator (Byers and Wood 1980, 1981; Byers et al. 1984). Such a short range effect could partially explain the inconsistency of verbenone in deterring attack by *D. ponderosae* on standing trees (Bentz et al. 1989; Lister et al. 1990; Gibson et al. 1991; Shea et al. 1992). High levels of verbenone could occur due to a combination of factors including spontaneous auto-oxidation of α -pinene as well as metabolic conversion by beetles and particularly microorganisms (Hunt et al. 1989; Hunt and Borden 1990). Therefore, a tree at an advanced stage of attack would exude verbenone at levels that would signal a limited fresh resource. The production of frontalin in high amounts by males as soon as they joined females strongly suggests that high concentrations of frontalin in synergism with verbenone may mask or neutralise the effect of the aggregation pheromones *exo*-brevicomin and *trans*-verbenol (Shore et al. 1992) and terminate aggregation.

2-Phenyl ethanol has been reported in *Ips* and *Dendroctonus* spp. and increased the attraction of *I. paraconfusus* to logs infested by males (Renwick 1976). Being a major component of aged onion pulp, it is a potent attractant for the onion fly, *Delia antiqua* (Meigen), the seed-corn fly, *D. platura* (Meigen) (Diptera: Anthomyiidae) and is a pheromone component

of many moths (Birch 1974; Kuwahara 1980; Ishikawa et al. 1983). It was detected in large amounts in the individual extracts of paired male *D. ponderosae* and reduced the attraction of beetles to attractant baited traps (Figure 6). It is a metabolite of phenylalanine in bark beetles and their yeasts (Ishikawa et al. 1983; Leufvén et al. 1984). As a fermentation product, it indicates advanced microbial activity (Ishikawa et al. 1983). My results suggest that it functions as an antiaggregation pheromone in *D. ponderosae*, probably synergising other antiaggregants.

Nonanal, produced by both sexes of *D. ponderosae* at all phases of attack deterred the capture of *I. pini* in pheromone-baited traps (Figure 7), indicating that it may be an interspecific synomone involved in resource partitioning. It is also part of a repellent blend of nonhost angiosperm bark volatiles that apparently aid *D. ponderosae* in rejecting unsuitable hosts (Borden et al. 1998) and as a normal constituent of lodgepole pine (Table 4), may be sequestered, rather than synthesised by bark beetles. Acetophenone was seen in both sexes at all phases of attack, in agreement with its presence in crushed abdomens of unfed and fed females (Pierce et al. 1987). Limonene oxide was present in low amounts in the volatiles of freshly emerged beetles and in the whole body extracts of some paired beetles. Limonene is abundant in lodgepole pine (Smith 1964, 1977), is released by *D. pseudotsugae* and synergises beetle produced pheromones (Rudinsky et al. 1977). Oxygenation of monocyclic terpenes like limonene, increases solubility in water and facilitates excretion (Leufvén and Birgersson 1986). *p*-Cymene was detected in the volatiles and extracts of emerged beetles. It was found in emergent females by Gries et al. (1990b) and is a metabolite of α -pinene, with *trans*-verbenol as an intermediate. Borneol, detected in low amounts in the volatiles of females, was reported previously in unmated virgin females (Libbey et al. 1985). Neither acetophenone, limonene oxide, *p*-cymene nor borneol had any effect on the capture of *D. ponderosae* in attractant baited traps when tested at release rates (Table 1) of 4.1-6.9 mg per 24 h.

These results did not disclose any new attractive pheromone component for *D. ponderosae* validating previous identifications. However, they indicate that termination of aggregation is not a simple phenomenon mediated by a single pheromone, verbenone. Rather, it is probably brought about by the changes in the ratios of *trans*-verbenol, *exo*-brevicommin, frontalin, 2-phenyl ethanol, verbenone and possibly nonanal, that emanate from the galleries as attack progresses.

4.1.2 *I. pini*

The trace amounts of ipsdienol in freshly emerged male *I. pini*, the dramatic increase when males bored into logs and the sharp decline in amount after they acquired females (Figures 3, 5) are consistent with observations on quantitative variation in aggregation pheromone production in male *I. typographus* (Birgersson et al. 1984). Similarly, there was a rapid decline in pheromone content following mating in *D. brevicomis*, *D. frontalis*, *I. paraconfusus*, *Ips calligraphus* (Germar) and *Scolytus multistriatus* (Marsham) (Peacock et al. 1971; Coster and Vité 1972; Hughes 1973a, 1973b; Elliott et al. 1975; Gore et al. 1977; Byers et al. 1981). This is probably the main factor in the termination of aggregation in *S. multistriatus*, *I. paraconfusus* (Gore et al. 1977; Byers 1981) and in *I. pini* as well.

Lanierone, produced in low amounts synergises ipsdienol in eastern (Teale et al. 1991) but not western (Miller et al. 1997) populations of *I. pini*. Therefore its presence in equal amounts in aerations of males at all phases and in high amounts in the individual extractions of emerged males suggests that it may be a vestigial trait. *cis*-Verbenol, *trans*-verbenol and verbenone which are known antiaggregants for *I. pini* (Miller 1990, Borden et al. 1991, Devlin and Borden 1994), were captured in the volatiles of emerged beetles and detected in the extracts of both sexes at all phases, suggesting a role in inducing dispersal in emerged beetles (Figure 5).

cis-Verbenol and verbenone may serve as antiaggregation pheromones that regulate attack density (Byers and Wood 1980; Byers 1983). All three volatiles are common in other bark beetles and could serve dual roles as pheromones and as interspecific synomones. *cis*-Verbenol was previously detected in feeding male *I. pini* (Vité et al. 1972); verbenone and *trans*-verbenol were identified in the volatiles of feeding females and *cis*-verbenol and myrtenol were detected in males (Cognato et al. 1997).

2-Phenyl ethanol was repellent to *D. ponderosae* in field tests (Figure 6). As it was present in the volatiles of beetles at all attack phases and in the extracts of males that were in the log alone, it is probably used as a synomone that mediates resource partitioning between the two species. The biological activity of 2-phenyl ethanol was long unknown (Ivarsson and Birgersson 1995). Gries et al. (1990a) detected its presence in extracts of wild and axenically reared *I. pini*. Derived from phenylalanine by the shikimic acid pathway, it is also a metabolite of yeasts associated with bark beetles (Leufvén et al. 1984; Gries et al. 1990a).

Acetophenone was present in all 40 emerged *I. pini* (Figure 5). 1-Phenyl ethanol is derived by its reduction (Pierce et al. 1987). Myrtenol, detected in the volatiles of freshly emerged beetles, is a product of metabolic oxidation or autoxidation of α -pinene (Hunt and Borden 1989). It was detected in the volatiles of *D. brevicomis* and *I. paraconfusus* (Byers 1983). In *D. frontalis*, it serves as a multifunctional pheromone, synergising the attraction of frontalin and *trans*-verbenol at low concentrations and contributing to antiaggregation at high concentrations (Rudinsky et al. 1974b). In *I. pini*, none of these compounds was behaviourally active.

Nonanal significantly inhibited the response of *I. pini* to ipsdienol (Figure 7). It is a ubiquitous compound found in the volatiles of lodgepole pine (Table 4) as well as in

D. ponderosae and *I. pini*. It is a constituent of trembling aspen bark, *Populus tremuloides* Michx, and in combination with 1-hexanol, benzyl alcohol and benzaldehyde from the same species, contributed to the disruption of aggregation in *D. ponderosae* (Borden et al. 1998). It is also an attractive host kairomone for many arthropods, e.g. the bird-cherry-oat aphid, *Rhopalosiphum padi* L., the desert locust, *Schistocerca gregaria* Forskal, the greater wax moth, *Galleria mellonella* (L.), the saw toothed grain beetle, *Oryzaephilus surinamensis* (L.), the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), the flat grain beetle, *C. pusillus* (Schonherr) and the tropical bont tick, *Amblyomma variegatum* F. (Pierce et al. 1990; Mushobozy et al. 1993; Turker et al. 1993; Steullet and Guerin 1994; Torto et al. 1996; Assad et al. 1997; Quiroz and Niemeyer 1998). Nonanal in the volatiles of plants acts as an allelochemic that inhibits seed germination (Bradow and Connick 1990) and probably serves in defence against pathogens and phytophagous insects due to its antifungal properties (Andersen et al. 1994) as well as cytotoxic and genotoxic effects (Eckl et al. 1993; Martelli et al. 1994). The effect of nonanal in decreasing polymerisation of brain microtubular protein (Miglietta et al. 1991), possibly renders it extremely toxic to *I. pini* which inhabits dead trees where toxic volatiles would be relatively low. Whether nonanal is sequestered or synthesised by *I. pini*, is as yet unknown.

exo- and *endo*-Brevicomin which are aggregation pheromones of *D. ponderosae* inhibited the response of *I. pini* to ipsdienol (Figure 7), contributing to the findings of Miller (1990) wherein a mixture of *exo*-brevicomin, *trans*-verbenol and ipsenol disrupted the response of *I. pini* to ipsdienol. This confirms that they are synomones that benefit both species in resource partitioning. The possible behavioural effects of the other antennally active compounds at concentrations other than those tested in this study cannot be ruled out without further investigation.

4.2 Interspecific interactions

The sympatric distribution of *D. ponderosae* and *I. pini*, their utilisation of a common resource, and the involvement of general metabolic pathways in the conversion of nutrients would account for common volatile compounds serving as semiochemicals (Hughes 1973a; White et al. 1980). Of the 16 compounds identified by GC-MS, seven were detected in the volatiles of both species (Table 2). Eleven of 12 compounds produced by *D. ponderosae* elicited an antennal response from *I. pini*, while 10 of 11 compounds produced by *I. pini* excited the antennae of *D. ponderosae*. The actual or hypothesised role of the 10 behaviourally active compounds is summarised in Table 11.

Mutual inhibition of attraction appears to be an important phenomenon in the attack dynamics of both species (Miller 1990; Safranyik et al. 1996). Antiaggregants present in both species on emergence would stimulate dispersal (Byers and Wood 1980; Byers 1983) and ensure outbreeding. Once a suitable host is located, aggregation pheromones produced by both species would recruit conspecifics to the tree (Pitman et al. 1969; Billings 1976; McKnight 1979; Borden et al. 1983; Conn et al. 1983; Libbey et al. 1985) and simultaneously repel heterospecifics (Miller 1990; Safranyik et al. 1996).

These semiochemicals would therefore serve multiple functions: attracting conspecifics, acquiring mates, preventing intersection with heterospecific galleries and ensuring resource partitioning along the bole – an elegant example of semiochemical parsimony (Blum 1996; Huber et al. 1999). Once the host resource is fully exploited and the tree is at an advanced stage of attack, aggregation would be terminated by an alteration in the ratio of antiaggregants to aggregants in *D. ponderosae* and a decline in the production of aggregation pheromone in *I. pini*.

Table 11 Actual or hypothesised role of behaviourally active compounds in this and other studies in the chemical ecology of *D. ponderosae* and *I. pini*

Producing species and compound	Producing sex	Responding species	Role of compound	References
<i>Dendroctonus ponderosae</i>				
<i>cis</i> -verbenol	female	both	Aggregation pheromone components for <i>D. ponderosae</i> . Inhibits response of <i>I. pini</i> to trees under attack by <i>D. ponderosae</i> .	Pitman et al. 1969; Billings 1976; Libbey et al. 1985; Miller 1990; Miller and Lafontaine 1992
<i>trans</i> -verbenol	female	both		
<i>exo</i> -brevicomin	male	both	Multifunctional pheromone for <i>D. ponderosae</i> attractive to conspecifics in low amounts, repellent in large amounts. Regulates attack. Inhibits response of <i>I. pini</i> (Figure 7).	McKnight 1979; Borden et al. 1983; Conn et al. 1983; Libbey et al. 1985.
<i>endo</i> -brevicomin	male	both	By-product in biosynthetic pathway of <i>exo</i> -brevicomin. Probably synergises aggregation of <i>D. ponderosae</i> . Inhibits response of <i>I. pini</i> (Figure 7).	Vanderwel 1994.
frontalin	male	<i>D. ponderosae</i>	Multifunctional pheromone. Attractive in small amounts, repellent in large amounts. Contributes to termination of attack on infested tree. Prevents gallery overlap, maintains male territory.	Rudinsky et al. 1974; Ryker and Libbey 1982.
verbenone	both	both	Antiaggregation pheromone and synomone for both species. Probably induces dispersal in freshly emerged beetles. Indicator of an advanced stage of attack. Keeps incoming <i>D. ponderosae</i> and <i>I. pini</i> away from attacked tree. Switches attack of <i>D. ponderosae</i> to adjacent trees.	Ryker and Yandell 1983; Miller 1990; Devlin and Borden 1994.
2-phenyl ethanol	both	<i>D. ponderosae</i>	Antiaggregant for <i>D. ponderosae</i> (Figure 6). Probable dispersal inducing agent in freshly emerged beetles. Synergises termination of attack.	continued...

Producing species and compound	Producing sex	Responding species	Role of compound	References
nonanal	both	<i>I. pini</i>	Inhibits response of <i>I. pini</i> to ipsdienol (Figure 7). Prevents intermingling of galleries. Helps in resource partitioning between species.	
<i>Ips pini</i>				
ipsdienol	male	both	Aggregation pheromone for <i>I. pini</i> . Inhibits response of <i>D. ponderosae</i> to its aggregation pheromones. Minimises interspecific competition.	Swaby and Rudinsky 1976; Birch et al. 1980; Lanier et al. 1980.
lanierone	male	<i>I. pini</i>	Synergises aggregation in <i>I. pini</i> . Does not appear to be a critical synergist in Western populations.	Teale et al. 1991; Miller et al. 1997.
<i>cis</i> -verbenol	both	both	Antiaggregation pheromones. Probably induce dispersal of emerged populations. Terminate aggregation after mate acquisition. Prevent intraspecific competition and overcrowding. May attract <i>D. ponderosae</i> to unoccupied tree and help overcome tree's defenses to enable easy colonisation by conspecifics	Miller 1990.
<i>trans</i> -verbenol	both	both		
verbenone	both	both		
nonanal	both	<i>I. pini</i>	Inhibits attraction of <i>I. pini</i> to ipsdienol (Figure 7). Possibly has a toxic effect. Possible antiaggregant sequestered from host. In combination with other antiaggregants, prevents overcrowding and terminates attack.	
2-phenyl ethanol	both	<i>D. ponderosae</i>	Inhibits attraction of <i>D. ponderosae</i> to its aggregation pheromone (Figure 6). Serves in resource partitioning and in minimising interspecific competition.	

4.3 Individual variation in pheromone production and its relationship with size

4.3.1 *D. ponderosae*

Size is an important fitness characteristic in bark beetles (Safranyik 1976; Sahota and Thompson 1979). Large body size confers enhanced survivorship, dispersal ability, pheromone production capacity and breeding opportunities (Reid 1962, 1963; Atkins 1967; McGhehey 1971; Thompson and Bennett 1971; Amman 1972; Safranyik 1976; Amman and Cole 1983; Gries 1985; Reid and Roitberg 1994; Robertson and Roitberg 1998). Based in part on my results, a hypothetical sequence of events portraying dispersal, host selection, mate choice, attack dynamics and reproductive success in *D. ponderosae* in relation to size, is depicted in Figure 18. At the beginning of colonisation, large females would initiate long galleries (Figure 12), but there would probably be no correlation between size and pheromone production in males (Figure 10). Therefore, any pheromone related criterion would be irrelevant in female choice. By not possessing the aggregation pheromone *trans*-verbenol on emergence and delaying production until they had bored into logs (Figure 4), female *D. ponderosae* would in fact be recruiting males of superior quality that had survived the dispersal phase, thereby indirectly exercising mate choice (Raffa et al. 1993). Mate choice may also depend on other behavioural criteria like stridulation involved in territoriality and rivalry in many *Dendroctonus* spp. (Rudinsky and Michael 1974; Rudinsky and Ryker 1976). Male guarding has been reported in *D. ponderosae* (McGhehey 1968). The first male to pair with a female in a new egg gallery guards the tunnel, stridulates when an intruder tries to enter, eventually meets him head to head and pushes him out of the gallery if he still persists (Fitzpatrick and Wellington 1983), making vigorous stridulation a criterion for female choice. Male choice may also exist, with high quality males selecting females in long galleries.

Figure 18. Hypothesised sequence of events in dispersal, host selection, mate choice, pheromone production dynamics and reproductive success in relation to size of adult *D. ponderosae*.

After pairing with a male, large females would produce significantly more of the antiaggregation pheromones verbenone and 2-phenyl ethanol than small ones (Figure 9). Males however, would quickly take over the production of antiaggregation pheromones, with large males producing copious amounts of *exo*-brevicomin, frontalin, verbenone and 2-phenyl ethanol (Figure 11). The amount of male produced antiaggregants would also increase with increasing female gallery length (Table 6).

Intraspecific resource partitioning and termination of aggregation are important in preventing overcrowding in *D. ponderosae*. At high population densities, intraspecific competition among bark beetles results in decline in body size, loss of vigour and increased mortality of emerging brood (Cole 1973; Beaver 1974; Amman and Pace 1976; Saarenmaa 1983). Small body size of brood larvae would be detrimental to the fitness of parents, as small female progeny would lay fewer eggs than large ones (Reid 1962, 1963; McGhehey 1971; Amman 1972; Amman and Cole 1983) and parent beetles that remain in the tree are smaller than those that re-emerge to establish second broods (Botterweg 1982; Robertson and Roitberg 1998).

Therefore, in *D. ponderosae*, reproductive success appears not to be related to the production of aggregation pheromone, but to the production of sufficient antiaggregation pheromone to prevent overcrowding, minimise intraspecific competition and to enable the production of large, fecund offspring. This would also explain the high correlation between various pheromones (Tables 5, 7). As hypothesised for aggregation pheromones (Borden et al. 1990), redundancy in the antiaggregation message would ensure response even by beetles with poor sensitivity to one or more components.

The role of a male bark beetle in a gallery with a female has often been questioned (Lissemore 1997). Most hypotheses suggest that males invest in their fitness by guarding against

predators and overtures from other males (Fitzpatrick and Wellington 1983; Kirkendall 1983; Lissemore 1997). My data for *D. ponderosae* now indicate that by taking over the task of pheromone production, males make a further investment in fitness that would allow females to invest metabolic energy in vitellogenesis, oviposition and brood rearing. The insignificant correlation between size and aggregation pheromone production at the beginning of colonisation (Figures 8, 10) suggests that the survival of individual beetles is more contingent on successfully attacking a tree than on producing large amounts of pheromone. At this early stage of attack, the survival of both the pheromone emitter and receiver is at stake. The male receiver therefore, will be primed to perceive minute amounts of pheromone and in turn most arriving males contain minute amounts of *exo*-brevicomin which synergises the attraction of *trans*-verbenol at low but not high concentrations (Borden 1987). Thus low amounts of aggregation pheromone should be sufficient to allow aggregation to occur. However, once an optimum attack density is reached, it is detrimental to the successful colonisers to recruit more beetles to the tree, as it will jeopardise their fitness (Cole 1973; Beaver 1974; Amman and Pace 1976; Saarenmaa 1983; Reid 1962; 1963; McGhehey 1971; Amman 1972; Botterweg 1982; Amman and Cole 1983; Robertson and Roitberg 1998). It will still be an advantage to dispersing beetles to colonise the attacked tree as they may yet find mates and reproduce – albeit with relatively few offspring. Thus a capacity for a rapid shift by pheromone emitters to release antiaggregants sufficient to deter receiving beetles would be highly adaptive. It is here therefore, that the true fitness of the male lies.

Although large males contained high amounts of antiaggregation pheromone, large females mated with males that were high antiaggregation pheromone producers, and large females are the most fecund, there was only a slight and insignificant trend towards size assortative mating in the field attacked tree (length: $r = 0.2239$, $P = 0.3427$; weight: $r = 0.2313$,

$P = 0.3264$), indicating that the largest males did not necessarily mate with the largest females. In a highly competitive environment, some prospecting males may accept small females that have successfully established a gallery, rather than risk rejecting a small female and investing further energy in seeking out a large one.

4.3.2 *I. pini*

Large male *I. pini* sire more offspring and have better breeding opportunities than small ones (Reid and Roitberg 1994; Robertson and Roitberg 1998). Females actively prospect for males during aggregation on a log and it is hypothesised that a strong pheromonal message released by large males would be a reliable indicator of male genetic quality (Miller et al. 1989; Reid and Stamps 1997). There was no significant relationship however, between ipsdienol production and male size, although a significant relationship did exist between size and the antiaggregant verbenone as well as 2-phenyl ethanol, which are repellent to *D. ponderosae* (Figure 14).

Angst and Lanier (1979) showed that the size of *I. pini* had no relationship with attraction either to the host or to ipsdienol. Size may therefore, be an indicator of general vigour of the beetle, but the amount of aggregation pheromone produced may not necessarily be associated with fitness in beetles that aggregate on dead trees. The question of whether aggregation on dead trees is at all beneficial was explored by Robins and Reid (1997), who showed that even at low densities, aggregation decreased the reproductive success of both male and female *I. pini*. Hence, it is not surprising to find a huge variation in the production of ipsdienol in individual beetles, with most producing relatively little pheromone and a few producing large amounts (Figure 14). This observation is consistent with a range of 0-2638 ng of ipsdienol found in male *I. pini* fed on lodgepole pine (Borden et al. 1986). The aggregative behaviour of bark beetles may enable weak pheromone producers to acquire mates and

reproduce (Schlyter and Birgersson 1989). The antennae of *I. pini* had low perception thresholds (0.01 µg) and very high saturation levels to ipsdienol compared to thresholds of 0.1 µg and 0.5 µg to the antiaggregants verbenone and *trans*-verbenol respectively, allowing for long distance detection and orientation towards low emissions of ipsdienol (Angst and Lanier 1979). Low ipsdienol producers might risk not being attractive to potential mates in return for not attracting ipsdienol responding predators and still achieve reproductive success (Borden et al. 1986; Miller et al. 1989; Schlyter and Birgersson 1989) accounting for the large number of low ipsdienol producers in the population. These relatively “silent” satellite males would probably also exploit the strong communication signal of a few high ipsdienol producers that has evolved because of a possible fitness effect to the signaller in attracting “legitimate receivers”, i.e. conspecific females (Otte 1972; Thornhill 1983). Satellite males are common in many insect groups in which aggregations are purely a consequence of a communication system intended to attract females to signalling males (Thornhill 1983), especially when aggregation on a scarce resource is of questionable benefit to pheromone producing males (Robins and Reid 1997), as it results in offspring of inferior quality (Rankin and Borden 1991).

Paternal investment is important in the reproductive success of *I. pini* (Kirkendall 1983; Reid and Roitberg 1994). While males may acquire multiple females, a female depends on a single male for the genes of her entire brood (Reid and Stamps 1997). Male choice is therefore important in the fitness of female *I. pini*. Although large males have high reproductive success (Reid and Roitberg 1994; Robertson and Roitberg 1998) and Reid (1992) inferred that female *I. pini* appeared to prefer large males, Teale et al. (1994) demonstrated that instead of being size based, assortative mating in *I. pini* was based on enantiomeric specificity. Therefore, enantiomeric specificity may be the primary determinant of mate choice, followed by some other unmeasured trait that may confer fitness (Teale et al. 1994). Ipsdienol production falls

sharply after males acquire females (Figures 3, 5). It is probable that after landing on a log, prospecting females orient towards patches emanating high concentrations of ipsdienol, a type of signal found in diverse species of animals, indicating that an area is occupied by unmated males (Lawrence 1987; Alatalo et al. 1992). Reid and Stamps (1997) deduced that female *I. pini* while choosing males, adjusted their acceptance threshold according to patch quality.

Pheromone production changes as a function of male mating status and female beetles would detect pheromones before landing at a breeding colony (Swaby and Rudinsky 1976; Byers 1981; Birgersson 1984). Once a female is in a desirable patch, she could further discriminate between males by finer non-olfactory criteria such as acoustic signals, diameter of the entrance hole or parasite load.

Prolonged attraction of males to females will jeopardise the reproductive success of both males and females (Kirkendall 1989). It is an advantage to the male to accept females within a short time frame, ensuring brood of the same age and thereafter ceasing to be attractive. Females prefer unmated males to those with one or more females (Reid and Stamps 1997), so male and female preferences may contribute to the short attraction period of a given male (Reid and Roitberg 1994). Significant positive relationships exist between female *trans*-verbenol production and size, both in freshly emerged and paired females (Figure 16, 17). There was also a significant increase in female *trans*-verbenol production with increasing gallery length ($r = 0.481$, $P = 0.0233$). In combination with the rapid cessation of ipsdienol production, after males acquire mates, female produced *trans*-verbenol may help terminate aggregation. Nonetheless, the rapid and complete shut down of ipsdienol production as soon as females are acquired is probably the major factor in the reproductive success of *I. pini*.

5. CONCLUSIONS

From the work presented in my thesis, the following conclusions can be drawn.

1. The two methods used for quantifying antennally active compounds do not provide completely comparable data. Capturing volatiles by aerating groups of beetles to death allowed the entire amount released by the beetles to be quantified and group aerations provided sufficient amount of compounds to enable GC-EAD analyses and identification. Extracting beetles by instant freezing and homogenisation allowed quantification of the amount of compound present at a given point in time and also made it possible to determine amounts in individual beetles.
2. GC-EAD analyses of volatiles from males and females of both species revealed the potential for a complex communication system within and between the two species, disclosing seven and nine compounds for *D. ponderosae* and *I. pini*, respectively, for which behavioural activity was not known.
3. 2-Phenyl ethanol produced by both species inhibited the response of *D. ponderosae* to its aggregation pheromone and therefore functions as an antiaggregation pheromone for *D. ponderosae* and as an interspecific synomone when released by *I. pini*.
4. Nonanal, detected in the volatiles of lodgepole pine and in both beetle species, significantly reduced the attraction of *I. pini* to ipsdienol. Thus, it would serve as an antiaggregant pheromone for *I. pini* and as a synomone when released by *D. ponderosae*.

5. *exo*- and *endo*-Brevicomin which are produced by *D. ponderosae*, significantly reduced the response of *I. pini* to ipsdienol, indicating their newly discovered function as synomones for both species.
6. The discovery of significant amounts of antiaggregation pheromones in freshly emerged beetles (verbenone in *D. ponderosae* and *cis*-verbenol, *trans*-verbenol and verbenone in *I. pini*) suggests that at this stage, these compounds would induce dispersal and ensure outbreeding of emergent brood.
7. In *D. ponderosae*, the surprising presence of large amounts of verbenone in females that were in the log alone suggests that successful aggregation depends on its influence being overpowered by aggregation pheromones during initiation of attack. In contrast, the sudden appearance of frontalin in paired males indicates that it, in combination with changes in the relative ratios of verbenone, *exo*-brevicomin and 2-phenyl ethanol would be the main factor in terminating aggregation.
8. In *I. pini*, the rapid cessation of ipsdienol production after females are acquired suggests that this would be the main factor in terminating aggregation. However, this effect would be facilitated by the production of *cis*-verbenol, *trans*-verbenol, verbenone and nonanal by both sexes. This would prevent overcrowding and ensure large, fecund offspring.
9. The high correlation between male size and production of antiaggregants in paired *D. ponderosae* and between female size and male antiaggregant production indicates that

pheromone mediated intraspecific resource partitioning and not the production of aggregation pheromone, is a major factor in reproductive success. Mate choice may involve other non-pheromonal cues such as gallery length and stridulation.

10. Male *I. pini* that produce very little ipsdienol would achieve reproductive success as “sneakers”. They would avoid the metabolic cost of pheromone production and would benefit by not attracting ipsdienol responding predators. They would acquire females by virtue of their aggregative behaviour, capitalising on the communication signal of the few high ipsdienol producers that attract females to the vicinity.

6. LITERATURE CITED

- Alatalo R.V., J. Hoglund, A. Lundberg and W.J. Sutherland. 1992. Evolution of black grouse leks: female preferences benefit males in larger leks. *Behav. Ecol.* 3: 53-59.
- Alcock J. 1982. Natural selection and communication among bark beetles. *Fla. Entomol.* 65: 1732.
- Amman G.D. 1972. Mountain pine beetle brood production in relation to thickness of lodgepole pine phloem. *J. Econ. Entomol.* 65: 138-40.
- Amman G.D. and V.E. Pace. 1976. Optimum egg gallery densities for the mountain pine beetle in relation to lodgepole pine phloem thickness. USDA For. Serv. Res. Note INT-209.
- Amman G.D. and W.E. Cole. 1983. Mountain pine beetle dynamics in lodgepole pine forests. Part II: population dynamics. USDA For. Serv. Gen. Tech. Rep. INT-145.
- Amman G.D. and L. Safranyik. 1985. Insects of lodgepole pine: Impacts and control. pp. 107-124. In: Baumgartner J.M., R.G. Krebill, J.T. Arnott and G.F. Weetman (eds.), *Lodgepole Pine, the Species and its Management*. Symposium Proceedings, May 14-16, 1984, Vancouver, BC, Canada, Washington State University, Cooperative Extension. 381 pp.
- Anderbrant O., F. Schlyter and G. Birgersson. 1985. Intraspecific competition affecting parents and offspring in the bark beetle *Ips typographus*. *Oikos*. 45: 89-98.
- Andersen R.A., T.R. Hamilton-Kemp, D.F. Hildebrand, C.T. McCrecken, Jr., R.W. Collins and P.D. Fleming. 1994. Structure-Antifungal activity relationships among volatiles C₆ and C₉ aliphatic aldehydes, ketones and alcohols. *J. Agric. Food Chem.* 42: 1563-1568.
- Angst M.E. and G.N. Lanier. 1979. Electroantennogram responses of two populations of *Ips pini* (Coleoptera: Scolytidae) to insect-produced and host tree compounds. *J. Chem. Ecol.* 5: 129-138.
- Arn H., E. Stadler and S. Rauscher. 1975. The electroantennographic detector – a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Z. Naturforsch.* 30c: 722-725.
- Ashraf M. and A.A. Berryman. 1969. Biology of *Scolytus ventralis* (Coleoptera: Scolytidae) attacking *Abies grandis* in northern Idaho. *Melanderia* 2. Washington State Entomological Society.
- Assad Y.O.H., A. Hassanali, B. Torto, H. Mahamat, N.H.H. Bashir and S.E. Bashir. 1997. *J. Chem. Ecol.* 23: 1373-1388.

- Atkins M.D. 1967. The effect of rearing temperature on the size and fat content of the Douglas-fir beetle. *Can. Entomol.* 99: 181-87.
- Beaver R.A. 1974. Intraspecific competition among bark beetle larvae (Coleoptera: Scolytidae). *J. Anim.Ecol.* 43: 455-467.
- Bentz B., C.K. Lister, J.M. Schmid, S.A. Mata, L.A. Rasmussen and D. Haneman. 1989. Does verbenone reduce mountain pine beetle attacks in susceptible stands of ponderosa pine? USDA, For. Ser., Res. Note RM-495.
- Berryman A.A. 1969. Response of *Abies grandis* to attack by *Scolytus ventralis* (Coleoptera: Scolytidae). *Can. Entomol.* 101: 1033-1042.
- Berryman A.A. 1972. Resistance of conifers to invasion by bark beetle fungal associations. *BioSci.* 22: 598-602.
- Berryman A.A. 1973. Population dynamics of the fir engraver, *Scolytus ventralis* (Coleoptera: Scolytidae). I. Analysis of population behaviour and survival from 1964 to 1971. *Can. Entomol.* 105: 1465-1488.
- Billings R.F., R.I. Gara and B.F. Hrutfiord. 1976. Influence of ponderosa pine resin volatiles on the response of *Dendroctonus ponderosae* to synthetic trans-verbenol. *Environ. Ent.* 5: 171-179.
- Birch M.C. 1974. Pheromones. North-Holland, Amsterdam.
- Birch M.C., D.M. Light, D.L. Wood, L.E. Brown, R.M. Silverstein, B.J. Bergot, G. Ohloff, J.R. West and J.C. Young. 1980. Pheromonal attraction and allomonal interruption of *Ips pini* in California by the two enantiomers of ipsdienol. *J. Chem. Ecol.* 6:703-717.
- Birgersson G.F., F. Schlyter, J. Lofqvist and G. Bergstrom. 1984. Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases. *J.Chem. Ecol.* 10:1029-1055.
- Birgersson G.F., F. Schlyter, G. Bergstrom and J. Lofqvist. 1988. Individual variation in aggregation pheromone content of the bark beetle, *Ips typographus*. *J. Chem. Ecol.* 14: 1737-1761.
- Blum M.S. 1996. Semiochemical parsimony in the arthropoda. *Annu. Rev. Entomol.* 41: 353-374.
- Borden J.H. 1975. Behavioural responses of coleoptera to pheromones, allomones and kairomones, pp. 169-198. In: H.H. Shorey and J.J. McKelvey, Jr. (eds.), *Chemical Control of Insect Behaviour: Theory and Application*. Wiley, New York.
- Borden J.H. 1984. Semiochemical-mediated aggregation and dispersal in the Coleoptera. In: Lewis, T. (ed). *Insect communication*. Acedemic Press, New York, pp. 123-149.

- Borden J.H. 1989. Semiochemicals and bark beetle populations: exploitation of natural phenomena by pest management strategists. *Holarctic. Ecol.* 12: 501-510.
- Borden J.H. 1995. Development and use of semiochemicals against bark and timber beetles. pp. 431-449. In: *Forest insects in Canada*. J.A. Armstrong and W.G.H. Ives (eds.). Natural Resources Canada, Ottawa.
- Borden J.H. 1997. Disruption of semiochemical-based aggregation in bark beetles (Coleoptera: Scolytidae). In: *Pheromone Research: New Directions*. R.T. Cardé and A.K. Minks (eds.). Chapman and Hall, New York.
- Borden J.H., D.W.A. Hunt, D.R. Miller and K.N. Slessor. 1986. Orientation in Forest Coleoptera: An uncertain outcome of responses by individual beetles to variable stimuli. In: Payne T.L. and M.C. Birch (eds.). *Mechanisms of Insect Olfaction*. Oxford University Press, New York.
- Borden J.H., J.E. Conn, L.M. Friskie, B.E. Scott, L.J. Chong, H.D. Pierce, Jr., B.D. Johnston and A.C. Oehlschlager. 1983. Semiochemicals for the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae), in British Columbia: Baited-tree studies. *Can. J. For. Res.* 13: 118-128.
- Borden J.H., L.C. Ryker, L.J. Chong, H.D. Pierce, Jr., B.D. Johnson and A.C. Oehlschlager. 1987. Response of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), to five semiochemicals in British Columbia lodgepole pine forests. *Can. J. For. Res.* 17: 118-128.
- Borden J.H., L.J. Chong and B.S. Lindgren 1990. Redundancy in the semiochemical message required to induce attack on lodgepole pines by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). *Can. Entomol.* 122: 769-777.
- Borden J.H., D.R. Devlin and D.R. Miller. 1991. Synomones of two sympatric species deter attack by the pine engraver, *Ips pini* (Coleoptera: Scolytidae). *Can J. For. Res.* 22: 381-387.
- Borden J.H., I.M. Wilson, R. Gries, L.J. Chong, H.D. Pierce, Jr. and G. Gries. 1998. Volatiles from the bark of trembling aspen, *Populus tremuloides* Michx. (Salicaceae) disrupt secondary attraction by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). *Chemoecology*. 8: 69-75.
- Botterweg P.F. 1982. Dispersal and flight behaviour of the spruce bark beetle *Ips typographus* in relation to sex, size and fat content. *Z. Angew Entomol.* 94: 466-489.
- Bradow J.M. and W.J. Connick. 1990. Volatile seed germination inhibitors from plant residues. *J. Chem. Ecol* 16: 645-667.
- Byers J.A. 1981. Effect of mating on terminating aggregation during host colonization in the bark beetle, *Ips paraconfusus*. *J. Chem. Ecol.* 7: 1135-1147.

- Byers J.A. 1983. Influence of sex, maturity and host substances on pheromones in the guts of the bark beetles, *Ips confusus* and *Dendroctonus brevicomis*. J. Insect Physiol. 29: 5-13.
- Byers J.A. 1989. Chemical ecology of bark beetles. Experientia 45: 271-283.
- Byers J.A. 1995. Host-tree chemistry affecting colonisation in bark beetles. pp. 154-213. In: Carde R.T. and W.J. Bell (eds). Chemical Ecology of Insects 2. Chapman and Hall, New York.
- Byers J.A. and D.L. Wood. 1980. Interspecific inhibition of the response of the bark beetles, *Dendroctonus brevicomis* LeConte and *Ips paraconfusus* Lanier, to their pheromones in the field. J. Chem. Ecol. 6:149-64.
- Byers J.A., D.L. Wood, J.C. Craig and L.B. Hendry. 1984. Attractive and inhibitory pheromones produced in the bark beetle, *Dendroctonus brevicomis*, during host colonization: Regulation of inter- and intraspecific competition. J. Chem. Ecol. 10(6) 861-877.
- Byrne K.J., W.E. Gore, G.T. Pearce and R.M. Silverstein. 1975. Porapak-Q collection of airborne organic compounds serving as models for insect pheromones. J. Chem. Ecol. 1: 1-7.
- Callaham R.Z. 1955. Oleoresin production in the resistance of ponderosa pine to bark beetles. Ph.D. dissertation, University of California, Berkeley. 120 pp., as cited in R.H. Smith, 1966. Resin quality as a factor in resistance of pines to bark beetles, pp. 189-196. In H.D. Gerhold, R.E. McDermott, E.J. Shriener and J.A. Winieski (eds.). Breeding pest-resistant trees. Proc. NATO NSF Symp., Pergammon Press, Oxford. 505 pp.
- Cole W.E. 1973. Crowding effects among single-age larvae of the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Environ. Entomol. 2: 285-293.
- Conn J.E., J.H. Borden, B.E. Scott, L.M. Friske, H.D. Pierce, Jr. and A.C. Oehlschlager. 1983. Semiochemicals for the mountain pine beetle in British Columbia: Field trapping studies. Can. J. For. Res. 13:320-324.
- Coster J.E. and J.P. Vité. 1972. Effects of feeding and mating on pheromone release in the southern pine beetle. Ann. Entomol. Soc. Am. 65:263-266.
- Day R.W. and G.P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr. 59: 433-463.
- Devlin D.R. and J.H. Borden. 1994. Efficacy of antiaggregants for the pine engraver, *Ips pini* (Coleoptera: Scolytidae). Can. J. For. Res. 24: 2469-2476.
- Dickens J.C., R.F. Billings and T.L. Payne. 1992. Green leaf volatiles interrupt aggregation pheromone response in bark beetles infesting southern pines. Experientia 48: 523-524.
- Eckl P.M., A. Ortner and H. Esterbauer. 1993. Genotoxic properties of 4-hydroxyalkenals and analogous aldehydes. Mut. Res. 290: 183-192.

- Elliott E.W., G.N. Lanier and J.B. Simone. 1975. Termination of aggregation by the European elm bark beetle, *Scolytus multistriatus*. J. Chem. Ecol. 1: 283-289.
- Fitzpatrick S.M. and W.G. Wellington. 1983. Insect territoriality. Can. J. Zool. 61: 471-486.
- Flamm R.O., R.N. Coulson, P. Beckley, P.E. Pulley and T.L. Wagner. 1989. Maintenance of a phloem-inhabiting guild. Environ. Entomol. 18: 381-387.
- Furniss, M.M. and V.M. Carolin 1977. Western Forest Insects. USDA For. Serv. Misc. Publ. No. 1339. pp 654.
- Furniss, M.M., V.F. Gallucci and R.I. Gara. 1980. Modelling the dynamics of mountain pine beetle aggregation in a lodgepole pine stand. Oecologia 46: 244-253.
- Furniss M.M., R.W. Clausen, G.P. Markin, M.D. McGregor and R.L. Livingstone. 1981. Effectiveness of Douglas-fir beetle antiaggregative pheromone applied by helicopter. USDA For. Serv. Gen. Tech. Rep. INT-101.
- Furniss M.M., G.P. Markin and Y.J. Hager. 1982. Aerial application of Douglas-fir beetle antiaggregative pheromone: equipment and evaluation. USDA For. Serv. Gen. Tech. Rep. INT-137.
- Geiszler D.R. and R.I. Gara. 1978. Mountain pine beetle attack dynamics in lodgepole pine. pp. 182-187. In: Berryman A.A., G.D. Amman and R.W. Stark (eds). Theory and Practice of Mountain Pine Beetle Management in Lodgepole Pine Forests. Univ. Idaho, Moscow and USDA, For. Serv., Washington, D.C.
- Geiszler D.R., V.F. Gallucci and R.I. Gara. 1980. Modelling dynamics of mountain pine beetle aggregation in a lodgepole pine stand. Oecologia 46: 244-253.
- Gibson K.E., R.F. Schmitz, G.D. Amman and R.D. Oakes. 1991. Mountain pine beetle response to different verbenone dosages in pine stands of western Montana. USDA, For. Serv. Res. Pap. INT-444.
- Gore W.E., G.T. Pearce, G.N. Lanier, J.B. Simeone, R.M. Silverstein, J.W. Peacock, R.A. Cuthbert. 1977. Aggregation attractant of the European elm bark beetle, *Scolytus multistriatus*: Production of individual components and related aggregation behaviour. J. Chem. Ecol. 3: 429-446.
- Gries G. 1985. Zur Frage der Dispersion des Buchdruckers (*Ips typographus* L.). Z. Angew. Entomol. 99: 12-20.
- Gries G., M.J. Smirle, A. Leufvén, D.R. Miller, J.H. Borden and H.S. Whitney. 1990a. Conversion of phenylalanine to toluene and 2-phenyl ethanol by the pine engraver *Ips pini* (Say) (Coleoptera: Scolytidae). Experientia 46: 329-331.

- Gries G., A. Leufvén, J.P. Lafontaine, H.D. Pierce, Jr. J.H. Borden, D. Vanderwel and A.C. Oehlschlager. 1990b. New metabolites of α -pinene produced by the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Insect Biochem. 20: 365-371.
- Gries G., R. Gries and J.H. Borden. 1992. 3,7,7-Trimethyl-1,3,5-cycloheptatriene in the volatiles of female mountain pine beetles, *Dendroctonus ponderosae*. Naturwissenschaften 79: 27-28.
- Hedden R.L. and G.B. Pitman. 1978. Attack density regulation: A new approach to the use of pheromones in Douglas-fir beetle population management. J. Econ. Entomol. 71: 633-637.
- Hopping G.R. 1961. Insects injurious to lodgepole pine in the Canadian Rocky Mountain Region in Alberta. pp. 77-87. In: Smithers, L.A. (ed.). Lodgepole Pine in Alberta. Can. Dep. For. Bull. No. 127.
- Huber D.P.W., R.Gries, J.H. Borden and H.D. Pierce, Jr. 1999. Two pheromones of coniferophagous bark beetles (Coleoptera: Scolytidae) found in the bark of nonhost angiosperms. J. Chem. Ecol. 25:805-816.
- Hughes P.R. 1973a. *Dendroctonus*: Production of pheromones and related compounds in response to host monoterpenes. Z. Angew. Entomol. 73: 294-312.
- Hughes P.R. 1973b. Effect of α -pinene exposure on *trans*-verbenol synthesis in *Dendroctonus ponderosae* Hopk. Experientia 60: 261-262.
- Hunt D.W.A., and J.H. Borden. 1989. Terpene alcohol pheromone production by *Dendroctonus ponderosae* and *Ips paraconfusus* (Coleoptera: Scolytidae) in the absence of readily culturable organisms. J. Chem. Ecol. 15: 1433-1463.
- Hunt D.W.A., and J.H. Borden. 1990. Conversion of verbenols to verbenone by yeasts isolated from *Dendroctonus ponderosae* (Coleoptera: Scolytidae). J. Chem. Ecol. 16: 1385-1397.
- Hunt D.W.A., J.H. Borden, H.D. Pierce, Jr., K.N. Slessor, G.G.S. King and E.K. Czyewska. 1986. Sex-specific production of ipsdienol and myrcenol by *Dendroctonus ponderosae* (Coleoptera: Scolytidae) exposed to myrcene vapours. J. Chem. Ecol. 12: 1579-1586.
- Hunt D.W.A., J.H. Borden and B.S. Lindgren. 1989. The role of auto-oxidation in the production of pheromones of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Can. J. For. Res. 19: 1275-1282.
- Ishikawa Y., T.I. Ikeshoji and Y. Matsumoto. 1983. 2-Phenyl ethanol: An attractant for the onion and seed-corn flies, *Hylemya antiqua* and *H. platura* (Diptera: Anthomyiidae). Appl. Entomol. Zool. 18: 270-277.
- Islas S.F. 1974. Observaciones sobre la biologica y el combate de los escarabajos descortezadores de les pinos: *Dendroctonus adjunctus* Blf. y *D. mexicanus* Hopk. en algunas regions del Estado de Mexico. Nat. Inst. For. Res., Mexico, D.F. Mexico. Tech. Bull. No. 40.

- Islas S.F., C.V. Ascencio and V.R. Muniz. 1981. Avances en la aplicacion del metodo de derribo y abandono. Proc. Sem. For. Res., 1981. Nat. Inst. For. Res., Mexico, D.F. Mexico.
- Ivarsson P. and G. Birgersson. 1995. Regulation and biosynthesis of pheromone components in the double spined bark beetle *Ips duplicatus* (Coleoptera: Scolytidae). J. Insect Physiol. 41: 843-849.
- Kirkendall L.R. 1983. The evolution of mating systems in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). Zool. J. Linn. Soc. 77: 293-352.
- Kuwahara Y. 1980. Isolation and identification of male-secreted possible sex pheromone from a pyralid moth, *Aphomia gularis* Zeller (Pyralidae: Lepidoptera). Appl. Entomol. Zool. 15: 478-485.
- Lanier G.N. and E.A. Cameron. 1969. Secondary sexual characters in the North American species of the genus *Ips* (Coleoptera: Scolytidae). Can. Entomol. 101: 862-870.
- Lanier G.N., A. Classon, T. Stewart, J.J. Piston and R.M. Silverstein. 1980. *Ips pini*: the basis for interpopulational differences in pheromone biology. J.Chem.Ecol. 6:677-687.
- Lawrence W.S. 1987. Dispersal: an alternative mating tactic conditional on sex ratio and body size. Behav. Ecol. and Sociobiol. 21: 367-363.
- Leufvén A. and G. Birgersson. 1986. Quantitative variation of different monoterpenes around the galleries of *Ips typographus* (Coleoptera: Scolytidae). Can. J. Bot. 65: 1038-1044.
- Leufvén A., G. Bergstrom and E. Falsen. 1984. Interconversion of verbenols and verbenone by identified yeasts from the spruce bark beetles *Ips typographus*. J. Chem. Ecol. 10: 1349-1361.
- Libbey L.M., L.C. Ryker and K.L. Yandell. 1985. Laboratory and field studies of volatiles released by *Dendroctonus ponderosae*. Z. Angew. Entomol. 100:381-392.
- Light D.M. and M.C. Birch 1979. Inhibition of attractive pheromone response in *Ips paraconfusus* by (R) - (-) – ipsdienol. Naturwissenschaften 66: 159-160.
- Lindgren. B.S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). Can. Entomol. 115: 229-302.
- Lindgren. B.S., J.H. Borden, G.H. Cushon, L.J. Chong and C.J. Higgins. 1989a. Reduction of mountain pine beetle (Coleoptera: Scolytidae) attacks by verbenone in lodgepole pine stands in British Columbia. Can. J. For. Res. 19: 65-68.
- Lindgren. B.S., M.D. McGregor, R.D. Oakes and H.E. Meyer. 1989b. Suppression of spruce beetle attacks by MCH released from bubble caps. West. J. App. For. 4: 49-52.
- Lissemore F.M. 1994. Sources of variation in male mating success and the function of frass-clearing by males in the pine engraver beetle *Ips pini* (Scolytidae) (Ph.D. thesis). Syracuse University, Syracuse, New York.

- Lissemore F.M. 1997. Frass clearing by male pine engraver beetles (*Ips pini*; Scolytidae): paternal care or paternity assurance? *Behav. Ecol.* 8: 318-325.
- Lister C.K., J.M. Schmid, S.A. Mata, D. Haneman, C. O'Neil, J. Pasek and L. Sower. 1990. Verbenone bubble caps ineffective as a preventive strategy against mountain pine beetle attacks in ponderosa pine. USDA, For. Serv. Res. Note RM-501.
- Livingstone R.L. 1979. The pine engraver, *Ips pini* (Say) in Idaho. Life history, habits and management recommendations. Idaho Dep. Lands For. Insect Dis. Contr. Rep.
- Lyon R.L. 1958. A useful secondary characteristic in *Dendroctonus* bark beetles. *Can. Entomol.* 60: 582-584.
- Martelli A., R. Canonero, M. Cavanna, M. Ceradelli, U.M. Marinari. 1994. Cytotoxic and genotoxic effects of five n-alkanals in primary cultures of rat and human hepatocytes. *Mut. Res.* 323: 121-126.
- McCambridge W.F. and F.B. Knight. 1972. Factors affecting spruce beetles during a small outbreak. *Ecology* 53: 830-839.
- McGhehey J.H. 1968. Territoriality behaviour of bark beetle males. *Can. Entomol.* 100: 1153.
- McGhehey J.H. 1971. Female size and egg production of the mountain pine beetle *Dendroctonus ponderosae* Hopkins – Nor. For. Res. Cent., Edmonton, Alberta. Inf. Rep. NOR-X-9.
- McGregor M.D., M.M. Furniss, R.D. Oakes, K.E. Gibson and H.E. Meyer. 1984. MCH pheromone for preventing Douglas-fir beetle infestation in windthrown trees. *J. For.* 82: 613-616.
- McKnight R.C. 1979. Differences in response among populations of *Dendroctonus ponderosae* Hopkins to its pheromone complex. M.Sc. thesis, University of Washington, Seattle, Washington.
- Miglietta A., A. Olivero, G. Gadoni and L. Gabriel. 1991. Effects of some aldehydes on brain microtubular protein. *Chem. –Biol. Interactions.* 78: 183-191.
- Miller D.R. 1990. Reproductive and ecological isolation: Community structure in the use of semiochemicals by pine bark beetles (Coleoptera: Scolytidae). Ph.D. thesis. Simon Fraser University, Burnaby, British Columbia.
- Miller D.R. and J.H. Borden. 1985. Life history of *Ips latidens* (LeConte) (Coleoptera: Scolytidae). *Can. Entomol.* 117: 859-871.
- Miller D.R. and J.H. Borden. 1990. β -Phellandrene: Kairomone for pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). *J. Chem. Ecol.* 16: 2519-2531.

- Miller D.R., J.H. Borden and K.N. Slessor. 1989. Inter- and intrapopulation variation of the pheromone ipsdienol produced by male pine engravers, *Ips pini* (Say) (Coleoptera: Scolytidae). J. Chem. Ecol. 15: 233-247.
- Miller D.R., J.H. Borden and B.S. Lindgren. 1995. Verbenone: dose-dependent interruption of pheromone-based attraction of three sympatric species of pine bark beetles. Environ. Entomol. 24: 692-696.
- Miller D.R., K.E. Gibson, K.F. Raffa, S.J. Seybold, S.A. Teale and D.L. Wood. 1997. Geographic variation in response of pine engraver, *Ips pini* and associated species to pheromone Lanierone. J. Chem. Ecol. 23: 2013-2031.
- Miller J.M. and F.P. Keen. 1960. Biology and control of the western pine beetle USDA For. Serv. Misc. Publ. No. 800.
- Moeck H.A. and C.S. Simmons. 1991. Primary attraction of mountain pine beetle, *Dendroctonus ponderosae* Hopk. (Coleoptera: Scolytidae), to bolts of lodgepole pine. Can. Entomol. 123: 299-304.
- Mushobozy D.K., H.D. Pierce, Jr. and J.H. Borden. 1993. Evaluation of 1-octen-3-ol and nonanal as adjuvants for aggregation pheromones for three species of cucujid beetles (Coleoptera: Cucujidae). J. Econ. Entomol. 86: 1835-1845.
- Otte D. 1972. Simple versus elaborate behaviour in grasshoppers: an analysis of communication in the genus *Syrbula*. Behaviour 42: 291-322.
- Paine T.D., M.C. Birch and P. Svihra. 1981. Niche breadth and resource partitioning by four sympatric species of bark beetles. (Coleoptera: Scolytidae). Oecologia 48: 1-6.
- Peacock J.W., A.C. Lincoln, J.B. Simeone and R.M. Siverstein. 1971. Attraction of *Scolytus multistriatus* to a virgin-female-produced pheromone in the field. Ann. Entomol. Soc. Am. 64: 1143-1149.
- Pierce A.M., H.D. Pierce, Jr., A.C. Oehlschlager and J.H. Borden. 1990. Attraction of *Oryzaephilus surinamensis* and *Oryzaephilus mercator* (Fauvel) (Coleoptera: Cucujidae) to some common volatiles of food. J. Chem. Ecol. 16: 465-475.
- Pierce H.D. Jr., J.E. Conn, A.C. Oehlschlager and J.H. Borden. 1987. Monoterpene metabolism in female mountain pine beetles, *Dendroctonus ponderosae* Hopkins, attacking lodgepole pine. J. Chem. Ecol. 13: 1455-1480.
- Pitman, G.B and J.P. Vité. 1969. Aggregation behaviour of *Dendroctonus ponderosae* in response to chemical messengers. Can. Entomol. 101: 143-149.
- Pitman G.B., J.P. Vité. G.W. Knizer, A.F. Fentiman Jr., 1968. Bark beetle attractants: *trans*-verbenol isolated from *Dendroctonus*. Nature. 218: 168-169.

- Pitman, J.B., J.P. Vité, G.W. Kinzer and A.F. Fentiman Jr. 1969. Specificity of population-aggregating pheromones in *Dendroctonus*. J. Insect Physiol. 15: 363-366.
- Poland T.M. and J.H. Borden. 1998a. Semiochemical-induced competition between *Dendroctonus rufipennis* and two secondary species, *Ips tridens* and *Dryocoetes affaber* (Coleoptera: Scolytidae). J. Econ. Entomol. 91: 1142-1149.
- Poland T.M. and J.H. Borden. 1998b. Competitive exclusion of *Dendroctonus rufipennis* induced by pheromones of *Ips tridens* and *Dryocoetes affaber* (Coleoptera: Scolytidae). J. Econ. Entomol. 91: 1150-1161.
- Quiroz A., and H.M. Niemeyer. 1998. Olfactometer-assessed responses of aphid *Rhopalosiphum padi* to wheat and oat volatiles. J. Chem. Ecol. 24: 113-124.
- Raffa K. 1988. Dynamics of forest insect populations: Patterns, causes, implications. In: A.A.Berryman (ed.). The Mountain Pine Beetle in Western North America. Plenum, New York.
- Raffa K.F. and A.A. Berryman. 1982. Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated microorganisms. Environ. Entomol. 11: 486-492.
- Raffa K.F. and A.A. Berryman. 1983. Physiological aspects of lodgepole pine wound responses to a fungal symbiont of the mountain pine beetle. Can. Entomol. 115: 723-734.
- Raffa K.F., T.W. Phillips and S.M. Salom. 1993. Strategies and mechanisms of host colonization by bark beetles. pp. 103-128. In: Beetle-Pathogen Interactions in Conifer Forests (eds.) T.D. Schowalter and G.M. Filip, Academic Press, New York.
- Rankin L.J. and J.H. Borden. 1991. Competitive interactions between the mountain pine beetle and the pine engraver in lodgepole pine. Can. J. For. Res. 21: 1029-1036.
- Reid M.L. 1992. Female mate choice and male parental care in a bark beetle, *Ips pini* (Coleoptera: Scolytidae). Ph.D. thesis, Simon Fraser University, Burnaby, British Columbia.
- Reid M.L. and B.D. Roitberg. 1994. Benefits of prolonged male residence with mates and brood in pine engravers (Coleoptera: Scolytidae). Oikos 70: 140-148.
- Reid M.L. and J.A. Stamps. 1997. Female mate choice tactics in a resource-based mating system: Field tests of alternative models. Am. Nat. 150: 98-121.
- Reid R.W. 1962. Biology of the mountain pine beetle, *Dendroctonus monticolae* Hopkins, in the east Kootenay region of British Columbia. I. Life cycle, brood development and flight periods. Can. Entomol. 94: 531-38.
- Reid R.W. 1963. Biology of the mountain pine beetle, *Dendroctonus monticolae* Hopkins, in east Kootenay region of British Columbia. III. Interaction between the beetle and its host, with emphasis on brood mortality and survival. Can. Entomol. 95: 225-238.

- Reid R.W., H.S. Whitney and J.A. Watson. 1967. Reactions of lodgepole pine to attack by *Dendroctonus ponderosae* Hopkins and blue stain fungi. Can. J. Bot. 45: 1115-1116.
- Renwick J.A.A., G.B. Pitman and J.P. Vité. 1976. 2-Phenyl ethanol isolated from bark beetles. Naturwissenschaften 63: 1968.
- Renwick J.A.A., and J.P. Vité. 1970. Systems of chemical communication in *Dendroctonus*. Contrib. Boyce Thompson Inst. 24: 283-292.
- Robertson I.C. and B.D. Roitberg. 1998. Duration of paternal care in pine engraver beetles: why do larger males care less? Beh. Ecol. Sociobiol. 43: 379-386.
- Robins G.L. and M.L. Reid. 1997. Effects of density on the reproductive success of pine engravers: is aggregation on dead trees beneficial? Ecol. Entomol. 22: 329-334.
- Rudinsky J.A. 1974. Additional components of the Douglas-fir beetle (Coleoptera: Scolytidae) aggregative pheromone and their utility in pest control. Z. Angew. Entomol. 76: 65-67.
- Rudinsky J.A. and R.R. Michael. 1974. Sound production in the Scolytidae: "rivalry" behaviour of male *Dendroctonus* beetles. J. Insect Physiol. 20: 1219-1230.
- Rudinsky J.A. and L.C. Ryker. 1976. Sound production in Scolytidae: rivalry and premating stridulation of male Douglas-fir beetle. J. Insect Physiol. 22: 997-1003.
- Rudinsky J.A., M.E. Morgan, L.M. Libbey and T.B. Putnam. 1974a. Additional compounds of the Douglas-fir beetle (Coleoptera: Scolytidae) aggregative pheromone and their possible utility in pest control. Z. Angew. Entomol. 76: 65-77.
- Rudinsky J.A., C. Sartwell, Jr., T.M. Graves and M.E. Morgan. 1974b. Granular formation of MCH: an antiaggregative pheromone of the Douglas-fir beetle and spruce bark beetles. Z. Angew. Entomol. 75: 254-263.
- Rudinsky J.A., L.C. Ryker, R.R. Michael, L.M. Libbey and M.E. Morgan. 1977. Olfactory and auditory signals mediating behavioural patterns of bark beetles. pp. 195-209. In: Labeyrie, V. (ed.), Comportement des insectes et milieu trophique, Coll. du C.N.R.S. Tours France, 13-17 Sept. 1976.
- Russell C.E. and A.A. Berryman. 1976. Host resistance to the fir engraver beetle. 1. Monoterpene composition of *Abies grandis* pitch blisters and fungus-infected wounds. Can. J. Bot. 54: 14-18.
- Ryker L.C. and L.M. Libbey. 1982. Frontalin in the male mountain pine beetle. J. Chem. Ecol. 8: 1399-1409.
- Ryker L.C. and J.A. Rudinsky. 1982. Field bioassay of *exo*- and *endo*-brevicomin with *Dendroctonus ponderosae* in lodgepole pine. J. Chem. Ecol. 8: 701-707.

- Ryker L.C. and K.L. Yandell. 1983. Effect of verbenone on aggregation of *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae) to synthetic attractant. *Z. Angew. Entomol.* 96: 452-459.
- Saarenmaa H. 1983. Modelling the spatial pattern and intraspecific competition in *Tomicus piniperda* (Coleoptera: Scolytidae). *Comm. Instit. For. Fenn.* 118: 1-40.
- Safranyik L. 1976. Size- and sex-related emergence and survival in cold storage of mountain pine beetles. *Can. Entomol.* 108: 209-212.
- Safranyik L. and D.A. Linton. 1991. Influence of competition on size, brood production and sex ratio in spruce beetles (Coleoptera: Scolytidae). *J. Entomol. Soc. Brit. Columbia.* 82: 52-55.
- Safranyik L., T.L. Shore and D.A. Linton. 1996. Ipsdienol and Lanierone increase *Ips pini* Say (Coleoptera: Scolytidae) attack and brood density in lodgepole pine infested by mountain pine beetle. *Can. Entomol.* 128: 199-207.
- Sahota T.S. and A.J. Thomson. 1979. Temperature induced variation in the rates of reproductive processes in *Dendroctonus rufipennis* (Coleoptera: Scolytidae): A new approach to detecting changes in population quality. *Can. Entomol.* 111: 1069-1078.
- Sartwell C., R.F. Schmitz and W.J. Buckhorn. 1971. Pine engraver, *Ips pini*, in the Western states. in the Western states. USDA, For. Serv. For. Pest Leaflet. No. 122.
- SAS Institute Inc. 1990. SAS/STAT® Users Guide, Release 6.03 Edition. Cary, North Carolina.
- Schenk J.A. and D.M. Benjamin. 1969. Notes on the biology of *Ips pini* in central Wisconsin jack pine forests. *Ann. Entomol. Soc. Am.* 62: 480-485.
- Schlyter F. and G. Birgersson. 1989. Individual variation in bark beetle and moth pheromones – a comparison and an evolutionary background. *Holarctic Ecol.* 12: 457-465.
- Schmitz R.F. 1972. Behaviour of *Ips pini* during mating, oviposition and larval development. (Coleoptera: Scolytidae). *Can. Entomol.* 104: 1723-1728.
- Schroeder. L.M. 1992. Olfactory recognition of nonhosts aspen and birch by conifer bark beetles *Tomicus piniperda* and *Hylurgops palliatus*. *J. Chem. Ecol.* 18: 1583-1593.
- Shea P.J., M.D. McGregor and G.E. Daterman. 1992. Aerial application of verbenone reduces attack of lodgepole pine by mountain pine beetle. *Can J. For. Res.* 22: 436-441.
- Shrimpton D.M. 1973. Extractives associated with the wound response of lodgepole pine attacked by the mountain pine beetle and associated micro-organisms. *Can. J. Bot.* 51: 527-534.
- Shore T.L., L. Safranyik and B.S. Lindgren. 1992. The response of mountain pine beetle (*Dendroctonus ponderosae*) to lodgepole pine baited with verbenone and *exo*-brevocomin. *J. Chem. Ecol.* 18: 533-541.

- Smith R.H. 1964. The monoterpenes of lodgepole pine oleoresin. *Phytochemistry*. 3: 259-262.
- Smith R.H. 1977. Monoterpenes of ponderosa pine xylem resin in western United States. USDA, For. Serv. Tech. Bull. No. 1532.
- Stark R.W. and J.H. Borden. 1965. Observations on mortality factors of the fir engraver beetle, *Scolytus ventralis* (Coleoptera: Scolytidae). *J. Econ. Entomol.* 58: 1162-1163.
- Steulett P. and P.M. Guerin. 1994. Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricus (Ixodidae). *J. Comp. Physiol. A.* 174: 27-38.
- Swaby, J.A. and J.A. Rudinsky. 1976. Acoustic and olfactory behaviour of *Ips pini* (Say) (Coleoptera: Scolytidae) during host invasion and colonization. *Z. Angew. Entomol.* 81: 421-432.
- Teale S.A., F.X. Webster, A. Zhang, and G.N. Lanier. 1991. Lanierone: a new pheromone component from *Ips pini* (Coleoptera: Scolytidae) in New York. *J. Chem. Ecol.* 17: 1159-1176.
- Teale S.A., B.J. Hager and F.X. Webster. 1994. Pheromone-based assortative mating in a bark beetle. *Anim. Behav.* 48: 569-578.
- Thomas J.B. 1961. The life history of *Ips pini* (Say). (Coleoptera: Scolytidae). *Can. Entomol.* 93: 384-390.
- Thompson S.N. and R.B. Bennett. 1971. Oxidation of fat during flight of male Douglas-fir beetles, *Dendroctonus pseudotsugae*. *J. Insect Physiol.* 17: 1555-1563.
- Thornhill, R. and J. Alcock. 1983. *The Evolution of Insect Mating Systems*. Harvard University Press, Cambridge, Massachusetts.
- Torto B., P.G.N. Njagi, A. Hassanali and H. Amiani. 1996. Aggregation pheromone system of nymphal gregarious desert locust, *Schistocerca gregaria* (Forsk.) *J. Chem. Ecol.* 22: 2273-2281.
- Turker L., I. Togan, S. Ergezen and M. Ozer. 1993. Novel attractants of *Galleria mellonella* (Lepidoptera: Pyralidae Galleriinae). *Apidologie* 24: 425-430.
- van den Dool H. and P.D. Kratz. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatog.* 2: 463-471.
- Van Sickle G.A. 1989. The status of mountain pine beetle in western Canada, 1988. pp. 6-8. In: G.D. Amman (ed.). *Proceedings-Symposium on the Management of Lodgepole Pine to Minimize Losses to the Mountain Pine Beetle*. USDA, For. Serv., Gen. Tech. Rep. INT-262.
- Vanderwel D. 1994. Factors affecting pheromone production in beetles. *Arch. Insect Biochem. Physiol.* 25:347-362.

- Vité J.P., A. Bakke and J.A.A. Renwick. 1972. Pheromones in *Ips* (Coleoptera: Scolytidae). Occurrence and production. Can. Entomol. 104: 1967-1975.
- White R.A., Jr., R. Agosin, R.T. Franklin, J.W. Webb. 1980. Bark beetle pheromones: Evidence for physiological synthesis mechanisms and the ecological implications. Z. Angew. Entomol. 90: 255-274.
- Whitney H.S. 1971. Association of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) with blue stain fungi and yeasts during brood development in lodgepole pine. Can. Entomol. 103: 1495-1503.
- Wilson I.M. 1995. Antiaggregants for the mountain pine beetle. Master of Pest Management thesis, Simon Fraser University, Burnaby, British Columbia.
- Wong B.L. and A.A. Berryman. 1977. Host resistance to the fir engraver beetle. 3. Lesion development and containment of infection by resistant *Abies grandis* inoculated with *Trichosporium symbioticum*. Can. J. Bot. 55: 2358-2365.
- Wood D.L. 1982. The role of pheromones, kairomones and allomones in the host selection and colonization of bark beetles. Annu. Rev. Entomol. 27: 411-446.
- Wright L.C.C., A.A. Berryman and S. Gurusiddaiah. 1979. Host resistance to the fir engraver beetle, *Scolytus ventralis* (Coleoptera: Scolytidae). 4. Effect of defoliation on wound monoterpene and inner bark carbohydrate concentrations. Can. Entomol. 111: 1255-1262.
- Zar J.H. 1984. Biostatistical Analysis, 2nd Edition. Prentice Hall, Engelwood Cliffs, New Jersey.