CHEMICAL ECOLOGY OF *PITYOGENES KNECHTELI* SWAINE (COLEOPTERA: SCOLYTIDAE) AND INTERACTIONS WITH OTHER SECONDARY BARK BEETLES

by

Anne Savoie

B.Sc. (Agr.), McGill University, 1993

THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF

MASTER OF PEST MANAGEMENT

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

April 1996

APPROVAL

Name: **ANNE SAVOIE**

Degree: **Master of Pest Management**

Title of Thesis:

CHEMICAL ECOLOGY OF *PITYOGENES KNECHTELI* SWAINE (COLEOPTERA:SCOLYTIDAE) AND INTERACTIONS WITH OTHER SECONDARY BARK BEETLES.

Examining Committee:

Chair: Dr. Z. Punja, Associate Professor

Dr. John Borden, Professor, Senior Supervisor
Department of Biological Sciences, SFU

Dr. H. D. Pierce, Research Associate
Department of Chemistry, SFU

Dr. L. Safranyik, Research Scientist
Pacific Forestry Centre

Dr. B. Roitberg, Professor
Department of Biological Sciences, SFU
Public Examiner

Date Approved 2 April 1996

PARTIAL COPYRIGHT LICENSE

I hereby grant to Simon Fraser University the right to lend my thesis, project or extended essay (the title of which is shown below) to users of the Simon Fraser University Library, and to make partial or single copies only for such users or in response to a request from the library of any other university, or other educational institution, on its own behalf or for one of its users. I further agree that permission for multiple copying of this work for scholarly purposes may be granted by me or the Dean of Graduate Studies. It is understood that copying or publication of this work for financial gain shall not be allowed without my written permission.

Title of Thesis/Project/Extended Essay

Chemical ecology of Pityogenes knechteli (Coleoptera: Scolytidae) and interactions with other secondary bark beetles

Author: _____
(signature)

Anne Savoie
(name)

10 April 1996
(date)

Abstract

The chemical ecology of *Pityogenes knechteli* Swaine, a secondary bark beetle which attacks lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann, in the British Columbia interior, was investigated. Three compounds found in male extracts, hexanol, (±)-ipsdienol and (*S*)-(-)-ipsenol, were identified as candidate pheromones, by use of coupled gas chromatographic-electroantennographic and mass spectrometric analyses. *Pityogenes knechteli* responded positively to (±)-ipsdienol and (*S*)-(-)-ipsenol in laboratory bioassays. In a field experiment, however, traps baited with (±)-ipsdienol alone caught 60% of all *P. knechteli* trapped, compared to < 10% for traps baited with (*S*)-(-)-ipsenol alone, and 23% for traps baited with both compounds. In other field trapping experiments, the addition of hexanol to (±)-ipsdienol or to (±)-ipsdienol plus (*S*)-(-)-ipsenol increased attraction at a very low release rate, but decreased attraction at high release rates. Ipsdienol is concluded to be the principal component of the aggregation pheromone for *P. knechteli.* It is hypothesized that ipsenol serves as a short-range attractant, whereas hexanol is a multifunctional pheromone contributing to attraction at low release rates, and preventing overpopulation of host at high release rates. My results lead to a general hypothesis regarding semiochemical-based interactions among four pine-infesting secondary bark beetles in British Columbia. *Ips pini* (Say), a more aggressive species which commonly inhabits the same hosts as *P. knechteli*, also uses ipsdienol as a pheromone. *Pityogenes knechteli* likely exploits ipsdienol produced by *I. pini* for long-range host

location, whereas ipsenol produced by *P. knechteli* would repel *I. pini* at close range, maintaining specificity between the two species. *Ips latidens* LeConte and *I. mexicanus* (Hopkins) also infest lodgepole pine, but not the same hosts as *P. knechteli* and *I. pini*. *Ips latidens* uses ipsenol as a pheromone and is repelled by ipsdienol. The pheromone for *I. mexicanus* is unknown; however, it was caught in traps baited with (±)-ipsdienol and (*S*)-(-)-ipsenol, suggesting that these two compounds are part of its pheromone blend. Ipsenol and ipsdienol thus serve as synomones maintaining species-specific communication and, together with host characteristics, contribute to resource partitioning and to avoidance of competition among the four species.

Acknowledgement

I thank H.D. Pierce Jr., R. Gries and G. Gries who provided invaluable assistance in the chemical aspects of this research. I thank E. vanRanden and J. Macias-Samano for their assistance in the field and the time they spent discussing ideas with me. I especially want to thank J.H. Borden for his assistance, persistence, and enthusiasm. He was always available to help me, and was ever encouraging and supportive.

I also want to thank L. Chong for the help she provided in the lab, as well as Brian Drobe from Weyerhaeuser Canada Ltd in Princeton for providing field sites. Finally, I want to thank R. Arès, S. Blatt, D. Hubert, R. McDonald, T. Poland, R. Setter, E. Tomlin, I. Wilson and several summer students for field assistance.

This research was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) Industrial Research Chair and Graduate Scholarship; the Canadian Forest Service; the Science Council of B.C.; the Council of Forest and Lumber Industries of B.C., Coast Forest and Lumber Sector; the Interior and Cariboo Lumber Manufacturers Associations; and seven forest industries.

Table of Contents

List of Tables

List of Figures

1. Introduction

Within a forest environment, trees of different species, age class and vigour, are subject to attack by numerous bark beetle species. These bark beetles interact with each other within their subcortical environment, either directly or indirectly. The interactions, both within and between species, are mediated in part by semiochemicals, including pheromones, kairomones, and synomones (Borden 1977, 1996; Nordlund 1981; D.L. Wood 1982).

Bark beetles which attack and kill trees of normal vigour are referred to as primary species, whereas secondary species generally attack dead or dying trees, and often trees previously attacked or co-attacked by primary species. Only at very high populations do secondary species attack and kill healthy trees (Rudinsky 1962; Furniss and Carolin 1977). Primary bark beetle species use aggregation pheromones to induce mass attack of a healthy host and thereby to overcome its resistance mechanisms (Borden 1974; Birch 1978). Secondary species also use aggregation pheromones, allowing them to locate and attack ephemeral hosts (Atkins 1966) and to colonize the available resource fully before it is taken over by a competing species (Birch 1984). Volatiles emanating from trees can serve as kairomones helping secondary species to locate or identify suitable hosts (Borden 1977, 1985; Byers 1989). Ethanol, for example, is an important kairomone because it foretells of anaerobic metabolism occurring in a moribund host (Moeck 1981; Klimetzek *et al.* 1986; Kelsey 1993, 1994).

Numerous species of bark beetles have similar habitat requirements, and may thus compete for the same resource. Some species can co-exist within the same host tree with no deleterious effects. Other species, however, avoid potentially detrimental competition by breeding in different hosts. Pheromones released by one species can be reciprocally perceived by members of a competing species, and thus often serve as mutually-beneficial synomones, indicating the presence of a potential competitor, and maintaining breeding isolation (Birch 1978, 1984; Borden 1996).

Two species breeding in the same host may exploit each other's pheromone. For example, *Pityogenes chalcographus* (L.) and *Ips typographus* (L.) typically breed in the same host, and *P. chalcographus* was attracted to a mixture of the synthetic pheromone of *I. typographus* (Benz *et al.* 1986; Zuber and Benz 1992; Byers 1993). However, the attraction of *I. typographus* to its own aggregation pheromone was significantly reduced by the presence of pheromone components of *P. chalcographus* (Byers 1993). The California five-spined ips, *Ips paraconfusus* Lanier, and the pine engraver, *I. pini* (Say), on the other hand, are almost never found breeding in the same host. Members of these two species are mutually inhibited by the other species' pheromone (Birch and Wood 1975). In the southern regions of the United States, four species of bark beetles, the southern pine beetle, *Dendroctonus frontalis* Zimmerman, and three *Ips* species, often inhabit the same host tree (Svihra *et al.* 1980; Paine *et al.* 1981). Birch *et al.* (1980b) found that *D. frontalis* is generally the first of the four species to attack, and that it is not attracted to logs occupied by any of the three *Ips* species. The three *Ips* species vary in their cross-attraction to the other

species. The result is that host trees are colonized very rapidly, and thus disadvantageous reproductive interactions are minimized.

Pityogenes knechteli Swaine is a secondary bark beetle species inhabiting lodgepole pines, *Pinus contorta* var. *latifolia* Engelmann, in British Columbia. It is commonly found breeding in small-diameter slash and thin-bark portions of trees previously attacked by *Dendroctonus* or *Ips* spp. At high population levels, it may attack green trees (Bright 1976; Furniss and Carolin 1977). Males are the pioneer sex, and each male is joined by five to eight females. *Pityogenes knechteli* has one or two generations per year. Larvae, pupae or adults overwinter, and adults emerging in spring can establish two broods (Reid 1955; Bright 1976). Two other *Pityogenes* species are found in British Columbia: *P. fossifrons* (LeConte) breeds in *Pinus monticola*, and *P. carinulatus* (LeConte) in *P. ponderosa*. *P. fossifrons* rarely attacks lodgepole pines (Bright 1976)

Several other secondary species, including *I. latidens* LeConte, *I. mexicanus* (Hopkins), and *I. pini*, infest lodgepole pine in the same environment as *P. knechteli*. *Ips pini* is one of the most common bark beetles in North America. It is associated with slash and weakened trees, and has up to two generations per year. Males initiate attack, and are joined by three or four females (Reid 1955; Furniss and Carolin 1977). The principal pheromone components for *I. pini* are (*S*)-(+)- and (*R*)-(-)-ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) which occur in varying ratios, depending on individual variation and geographic location (Miller *et al.* 1989, 1996; Seybold *et al.* 1995). A secondary pheromone component that varies in potency with geographic

location and season is lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one) (Teale *et al.* 1991; Seybold *et al.* 1992). *Ips latidens* generally attacks the tops and limbs of dead, dying, or weakened trees (Bright 1976). Males initiate most attacks. In south-central British Columbia, *I. latidens* has a single generation per year with a partial second brood (Miller and Borden 1985). Its pheromone consists of ipsenol (2-methyl-6-methylene-7-octen-4-ol) of unknown chirality, although males were found to show a slight preference for the (*S*)-(-) enantiomer (Miller *et al.* 1991). *Ips mexicanus* attacks the bole of living, injured, dying and recently downed trees, and is usually associated with other bark beetles. It has up to three generations per year in California, but probably only one in the northern part of its range (Furniss and Carolin 1977). Its pheromone is unknown, although Miller *et al.* (1991) captured it in traps baited with ipsenol.

Miller (1991) examined the semiochemical-based interactions between sympatric pine bark beetles in British Columbia, including *I. pini* and *I. latidens*. He found that ipsenol, which is produced by *I. latidens*, repels *I. pini*, and that the attraction of *I. latidens* to its own pheromone is inhibited by (*S*)-(+)-ipsdienol produced by *I. pini*. The two species do not co-inhabit the same host tree (Miller and Borden 1992). Poland (1993) studied the interactions between *I. pini* and *P. knechteli*, species that do breed within the same host tree. The two species appear to partition the shared phloem resource based on tree diameter, with *I. pini* associated with large-diameter hosts, and *P. knechteli* with small-diameter hosts (Poland and Borden 1994a). Similar results were reported for *P. chalcographus* and *I. typographus* in Norway

spruce, *Picea abies* (L.) (Benz *et al.* 1986). Poland and Borden (1994b) also found that *P. knechteli* males produce an aggregation pheromone that is attractive to both sexes. They concluded that the two species are not strongly competitive, and that they exploit each other's pheromone in host location.

A full understanding of the role of the pheromone components of a specific species can only be achieved by studying the species in the context of the ecosystem in which it lives, rather than in isolation (Birch 1978, 1984). Therefore, my objectives were to identify the aggregation pheromone components produced by male *P. knechteli*, to characterize the response by *P. knechteli* to the synthetic compounds in the laboratory and in the field, and to explore the semiochemical-based interactions between *P. knechteli* and three other secondary species co-existing with it in the lodgepole pine forest.

2. Methods and Materials

2.1 General Procedures

Bolts from slash or windthrown lodgepole pine trees naturally infested with *P. knechteli* were collected in late fall 1993 and in the spring and fall 1994 near Princeton, B.C. The ends of the bolts were waxed to prevent desiccation, and the bolts were stored at 5^{0}C until needed. Fresh, uninfested trees were also felled and cut into bolts. The ends were waxed and the bolts were kept under shade until needed. Bolts infested with beetles were transferred periodically into rearing cages held at approximately 27^{0}C. Emergent beetles were sexed and starved for 24-48 h on moist filter paper before they were used.

Candidate attractants were tested in the laboratory using walking beetles in a modified open arena olfactometer (Wood and Bushing 1963; Stock and Borden 1983). Beetles in groups of 10 were released 10 cm downwind of the exit port of a 10.0 mm ID glass tube containing a rolled 42.5 mm diam. filter paper on which 10 μL of pentane (control stimulus) or a pentane solution of candidate volatiles had been placed. Air passed through the tube at 350 mL per min, and beetles were given 2 min to respond. Positive responders, who entered a 2.0 x 2.0 x 3.0 cm triangular area directly downwind of the exit port, were removed immediately. After each trial, the beetles were mixed with beetles from other groups. Each insect was used no more than twice a day with a minimum of 2 h between tests. Males and females were tested separately. A complete series of bioassays for an experiment was done on the

same day to avoid any variation in response between days. To prevent departure of walking beetles, the arena was enclosed with a fine mesh screen, and red lighting was used to minimize spontaneous flight.

Field trapping experiments were conducted in 1994 and 1995 in a lodgepole pine forest located 30 km east of Princeton, B.C. Twelve-unit multiple funnel traps (Lindgren 1983) were placed 15 m apart in a randomized complete block design consisting of 10 blocks for each experiment. The traps were placed 10-15 m inside the forest margin. All chemicals, sources, release devices, and release rates are listed in Table 1.

2.2 Isolation and Preliminary Identification of *P. knechteli* Pheromone

Approximately 500 male and 500 female *P. knechteli* were confined in gel capsules on separate uninfested logs cut from the same tree. They were allowed to bore into the bark for 24 to 48 h, and were then excised from the logs and crushed over dry ice in a 95:5 pentane:ether solution (10 μL / beetle). The extracts were tested for attractiveness in laboratory Bioassay Experiment 1 (see Table 2 for treatments). Due to low numbers of male beetles, only females were used. Five groups of 10 beetles were tested.

Table 1. Description of semiochemicals and methods of their deployment in Field Exp. 1-6.

Semiochemical	Source[a]	Purity[b]	Release Device	Release Rate (mg per 24 h)	Field Exp. No
(±)-ipsdienol	P	97% chemical	bubble cap	0.2	1-6
(*S*)-(+)-ipsdienol	P	97% chemical 98% optical	bubble cap	0.2	3
(*R*)-(-)-ipsdienol	P	97% chemical 98% optical	bubble cap	0.2	3
(±)-ipsenol	P	99% chemical	bubble cap	0.2	2
(*S*)-(-)-ipsenol	P	96% chemical 97% optical	bubble cap	0.2	4,6
very low hexanol	S	98%	microcentrifuge tube (250 µL)	0.5[c]	6
low hexanol	S	98%	closed polyethylene bottle (15 mL) with hole	3[c]	1,2,3
low hexanol	S	98%	bubble cap	3.8	5,6
intermediate hexanol	S	98%	open polyethylene bottle (15 mL)	15[c]	5
high hexanol	S	98%	open polyethylene bottle with soaked wick	300[c]	5

[a] Symbols for sources as follows: P = Phero Tech Inc., Delta, B.C.; S = Sigma Chemical Company, St-Louis. Mo.

[b] Purity as listed by manufacturer.

[c] Determined in laboratory at 24° C. All other release rates determined by Phero Tech, Inc. at 22° C.

Coupled gas chromatographic-electroantennographic analyses (GC-EAD) (Arn *et al.* 1975), modified for processing small insects (Gries 1995) were performed on 10 newly emerged females to identify potential pheromone components in the male extract. The apparatus used a Varian 3400 gas chromatograph fitted with a DB-5-coated, fused silica column (30 m x 0.32 mm ID). The identities of antennally-active compounds were confirmed by coupled GC-mass spectrometry (MS) in electron impact mode (EI) using a DB-5 column in a Varian Saturn 3400 ion trap instrument. Synthetic candidate pheromones were further subjected to GC-EAD analyses to compare their EAD activity with those of male-produced compounds.

Three male-produced compounds which elicited responses from female antennae were tested in two field experiments. Field Exp. 1 tested the attractiveness of (±)-ipsdienol and hexanol alone and combined. Treatments were: 1) unbaited control traps; 2) (±)- ipsdienol; 3) hexanol; and 4) (±)-ipsdienol plus hexanol. Field Exp. 2 compared the attractiveness of (±)-ipsenol alone and in combination with (±)-ipsdienol and hexanol. Treatments were: 1) unbaited control traps; 2) (±)-ipsenol; 3) (±)-ipsdienol plus hexanol ; 4) (±)-ipsenol plus (±)-ipsdienol plus hexanol.

2.3 Enantiomeric Specificity of Ipsenol and Ipsdienol

Enantiomeric composition of ipsenol and ipsdienol in extracts was determined by GC-MS (Hewlett Packard 5985B) in selected-ion monitoring (SIM) mode using a Cyclodex-B column (30 m x 0.25 mm ID), with isobutane for chemical ionization (CI). Synthetic compounds, a hexane blank, and a concentrated pheromone

extract were analyzed.

Three laboratory bioassay experiments (Bioassay Exp. 2-4) were conducted to determine effective doses of, and interactions between, ipsenol and ipsdienol of natural chirality. Four groups of 10 beetles of each sex were tested for each treatment. See Table 2 for treatments.

The attractiveness of ipsdienol enantiomers was tested in Field Exp. 3. Treatments were: 1) unbaited control traps; 2) (±)-ipsdienol plus hexanol; 3) (*S*)-(+)-ipsdienol plus hexanol; and 4) (*R*)-(-)-ipsdienol plus hexanol. Field Exp. 4 tested the attractiveness of (*S*)-(-)-ipsenol and (±)-ipsdienol, alone or in combination. Treatments were: 1) unbaited control traps; 2) (*S*)-(-)-ipsenol; 3) (±)-ipsdienol; and 4) (*S*)-(-)-ipsenol plus (±)-ipsdienol.

2.4 Role of Hexanol

Two more laboratory bioassay experiments were conducted to test the effect of adding low and high doses of hexanol to ipsenol and ipsdienol. See Table 2 for treatments.

Ipsdienol was tested in combination with hexanol released at low, intermediate and high rates in Field Exp. 5. Treatments were: 1) unbaited control traps; 2) (±)-ipsdienol; 3) (±)-ipsdienol plus low hexanol; 4) (±)-ipsdienol plus intermediate hexanol; and 5) (±)-ipsdienol plus high hexanol. Field Exp. 6 examined the effect of adding hexanol to (*S*)-(-)-ipsenol plus (±)-ipsdienol. Treatments were: 1) unbaited control traps; 2) (*S*)-(-)-ipsenol plus (±)-ipsdienol; 3) (*S*)-(-)-ipsenol plus

(±)-ipsdienol plus very low hexanol; and 4) (*S*)-(-)-ipsenol plus (±)-ipsdienol plus low hexanol.

2.5 Statistical Analysis

Chi-square analysis was performed on the laboratory bioassay results, wherein the response by the pooled test population for each treatment was compared with that of the control population (see Stock 1981 for validation of procedure). The results from the field experiments were analyzed by two-way analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute 1994) and the means were compared using the Ryan-Einot-Gabriel-Welsch multiple-range test (REGWF) (Day and Quinn 1989). The numbers of beetles caught in the traps were transformed to $X' = \log (X+1)$ to stabilize the variance and normalize the data if needed (Zar 1984).

3. Results

3.1 Pheromone Components

In Bioassay Exp. 1, female *P. knechteli* were highly attracted to pentane extracts of males at a dose of 1.0 male eq; no other stimulus elicited a significant response (Table 2). This result justified further analysis of male extracts. Three male-produced compounds elicited an antennal response in females (Fig. 1). Retention indices of the three compounds suggested that they were hexanol, ipsenol and ipsdienol. The identity of the compounds was confirmed using GC-MS-EI by comparison of their spectra to that of authentic samples. Synthetic and male-produced compounds elicited similar antennal responses (Fig. 1).

Females in Field Exp. 1 were captured at a significant level only to traps baited with the combination of (±)-ipsdienol and hexanol (Fig. 2). Males did not respond to any treatment. Addition of (±)-ipsenol to (±)-ipsdienol plus hexanol caused no significant change in the numbers of beetles captured. (±)-Ipsenol alone was unattractive (Fig. 2, Field Exp. 2)

Table 2. Response of *Pityogenes knechteli* in Bioassay Exp. 1-6. Fifty females tested per stimulus in Bioassay Exp. 1 and 40 beetles of each sex tested in Bioassay Exp. 2-6.

Bioassay Exp. No.	Stimulus	Dose	Percent Response [a]	
			Males	Females
1	pentane	10 µL	-	16.0
	female extract	0.1 eq.	-	12.0
		1.0 eq.	-	20.0
	male extract	0.1 eq.	-	32.0
		1.0 eq.	-	58.0*
2	pentane	10 µL	10.0	15.0
	(*S*)-(-)-ipsenol	0.001 ng	12.5	15.0
		0.01 ng	5.0	40.0*
		0.1 ng	20.0	30.0
		1.0 ng	37.5*	30.0
		10.0 ng	22.5	25.0
		100.0 ng	10.0	7.5
3	pentane	10 µL	17.5	17.5
	(±)-ipsdienol	0.001 ng	37.5*	32.5
		0.01 ng	37.5*	22.5
		0.1 ng	22.5	37.5*
		1.0 ng	30.0	37.5*
		10.0 ng	12.5	25.0
		100.0 ng	27.5	17.5

Bioassay Exp. No.	Stimulus	Dose	Percent Response [a]	
			Male	Female
4	pentane	10 μL	17.5	15.0
	(*S*)-(-)-ipsenol	1.0 ng	27.5	20.0
	(*S*)-(-)-ipsenol:(±)-ipsdienol	0.1: 0.1 ng	25.0	32.5
		1.0 : 0.1 ng	22.5	30.0
		1.0 : 1.0 ng	40.0*	37.5*
5	pentane	10 μL	15.0	12.5
	(*S*)-(-)-ipsenol:(±)-ipsdienol	1.0 : 1.0 ng	35.0*	32.5*
	(*S*)-(-)-ipsenol:(±)-ipsdienol:hexanol	1.0:1.0:0.01ng	30.0	25.0
		1.0:1.0:0.1 ng	22.5	25.0
		1.0:1.0:1.0 ng	22.5	12.5
6	pentane	10 μL	15.0	20.0
	(*S*)-(-)-ipsenol:(±)-ipsdienol	1.0 : 1.0 ng	35.0*	47.5*
	(*S*)-(-)-ipsenol:(±)-ipsdienol:hexanol	1.0:1.0:1.0 ng	22.5	30.0
		1.0:1.0:10 ng	22.5	10.0
		1.0:1.0:100 ng	27.5	5.0*

[a] Asterisk indicates significant difference from response to pentane control, Chi-square test, $P < 0.05$.

Figure 1. Gas chromatograms of male extract (top) and of a standard solution consisting of hexanol, (±)-ipsdienol and (±)-ipsenol in a 25:1:1 ratio (middle). A female *Pityogenes knechteli* antenna was used in the electroantennographic detector (EAD) (bottom).

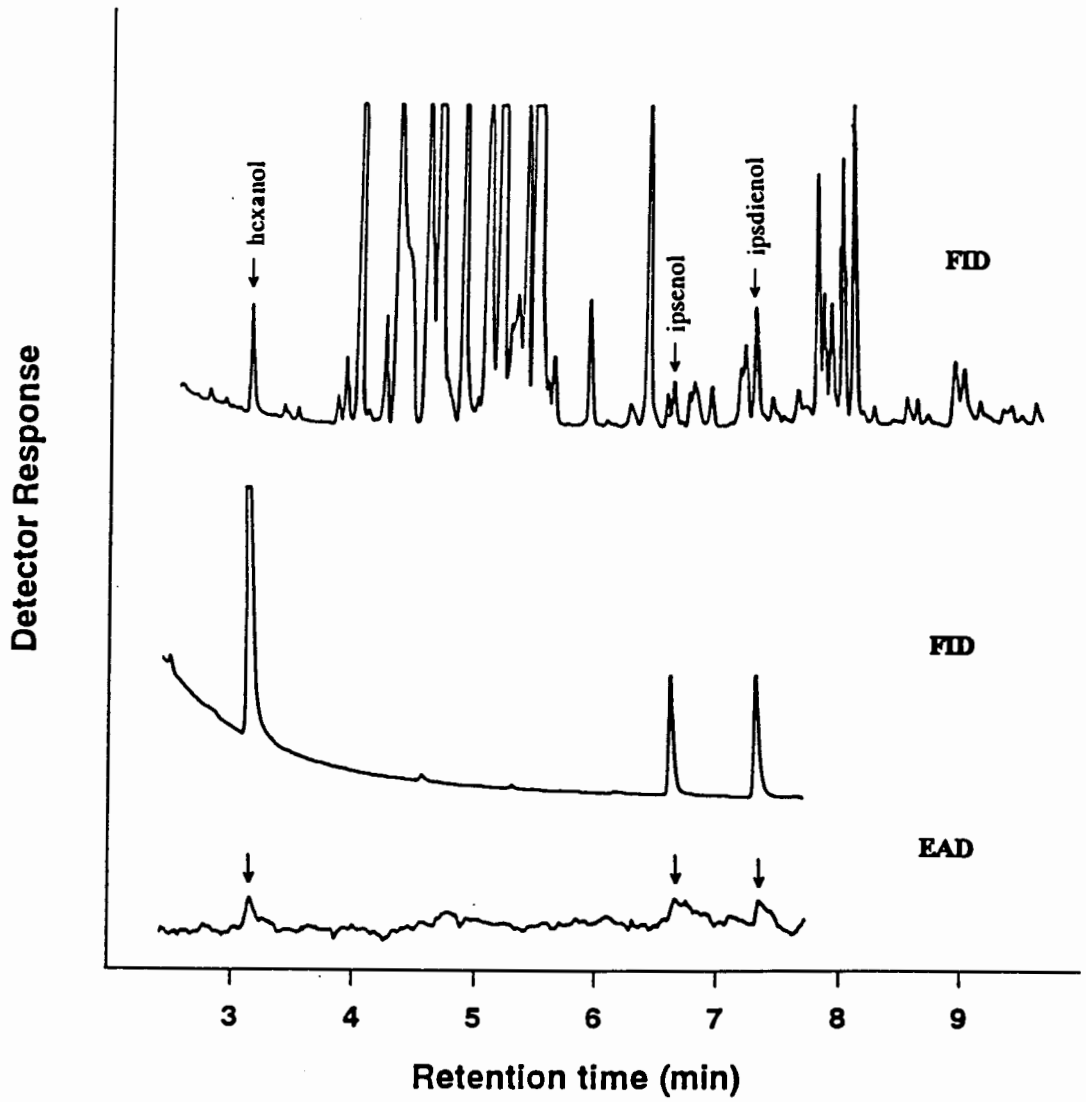
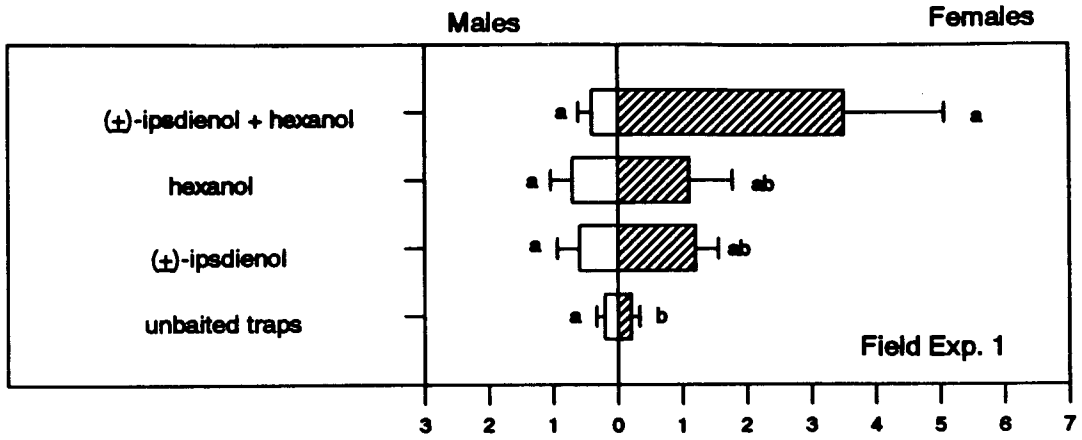
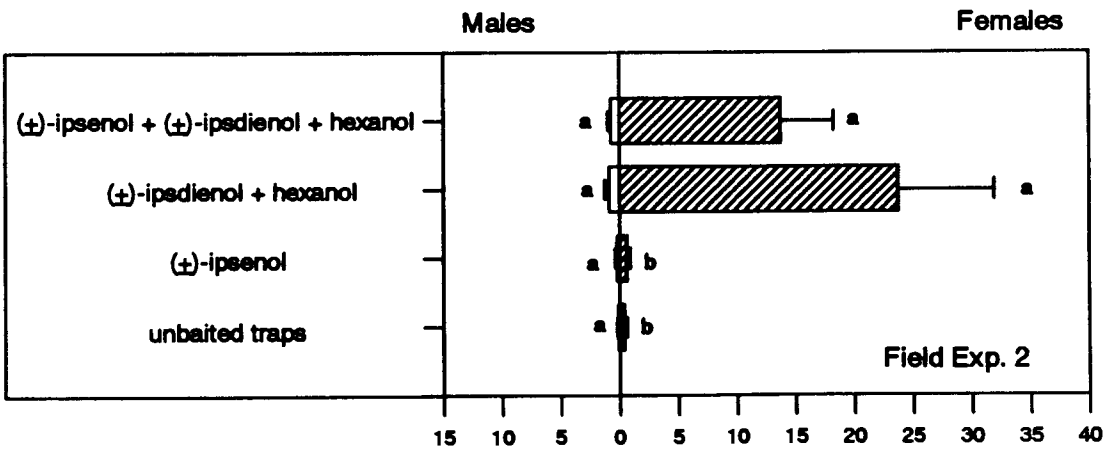

hexanol
ipsenol
ipsdienol
FID
FID
EAD
Detector Response
3
4
5
6
7
8
9
Retention time (min)

Figure 2. Number of *Pityogenes knechteli* caught in multiple funnel traps, in spring 1994 (Field Exp. 1) and in fall 1994 (Field Exp. 2). Within an experiment, bars with the same letter for each sex are not significantly different, two-way ANOVA and REGWF test on data transformed by $X' = \log (X + 1)$, $P < 0.05$. In both experiments, hexanol was released at a rate of approximately 3 mg per 24 h.





Number of Beetles Caught (mean + SE)

3.2 Enantiomeric Specificity

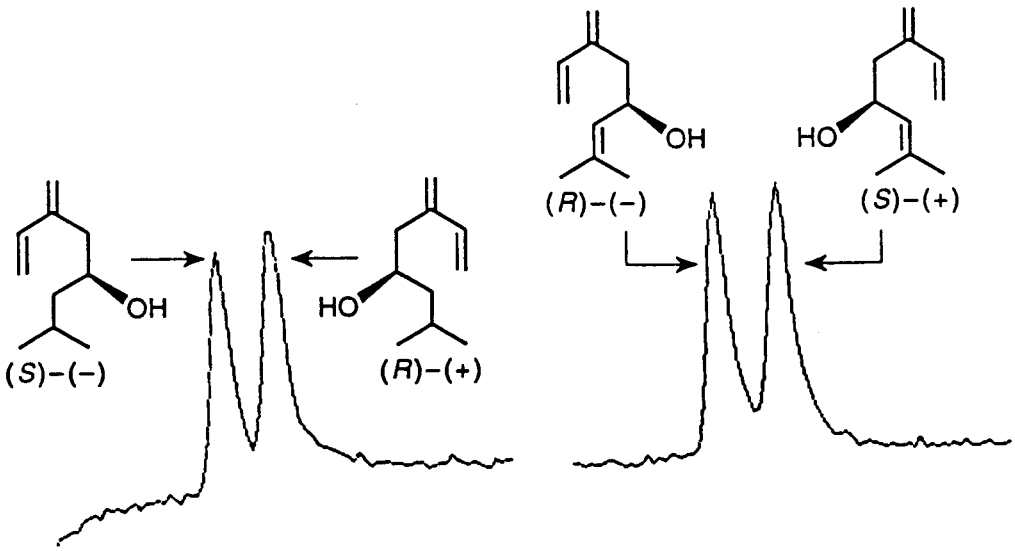
Analyses by GC-MS-CI-SIM of male extract and of synthetic ipsdienol and ipsenol on the chiral column resulted in retention time and ion ratio matches of synthetic and male-produced ipsdienol. Both (*S*)-(+)- and (*R*)-(-)-ipsdienol are produced by the beetle (Fig. 3). The ion ratio of synthetic and male-produced ipsenol, however, did not match. This result could be due to the presence of another compound contributing to one ion, and therefore distorting the ratio. The quantity of extract available was too small for further analyses to be conducted, and it cannot be concluded for certain whether male *P. knechteli* produce ipsenol. A compound eluting at approximately the same retention time as ipsenol, and having characteristic ions for ipsenol, suggested that the (*S*)-(-)- enantiomer might be present in the extract (Fig. 3).

In Bioassay Exp. 2, males and females were significantly attracted to (*S*)-(-)-ipsenol, but only at doses of 1.0 and 0.01 ng, respectively (Table 2). (±)-Ipsdienol at 0.001 and 0.01 ng doses was attractive to males, and females responded positively to (±)-ipsdienol at doses of 0.1 and 1.0 ng (Table 2, Bioassay Exp. 3). When both (*S*)-(-)-ipsenol and (±)-ipsdienol were tested together (Bioassay Exp. 4) the 1.0:1.0 ng combination was the most attractive for both males and females (Table 2).

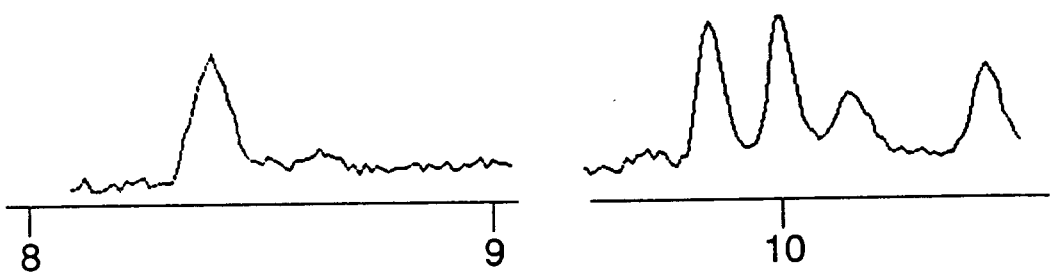
In Field Exp. 3, *P. knechteli* males and females responded significantly in equivalent numbers only to traps with (±)- or (*S*)-(+)-ipsdienol as part of the stimulus (Fig. 4). (*R*)-(-)-ipsdienol was neither attractive nor inhibitory. When (*S*)-(-)-ipsenol and (±)-ipsdienol were tested alone and in combination in Field Exp. 4, only (±)-ipsdienol was attractive to both males and females (Fig. 4). The presence of

Figure 3. Selected ion chromatograms (*m/z* 137 and *m/z* 155 for ipsenol, and *m/z* 135 and *m/z* 136 for ipsdienol) of synthetic (top) and beetle-produced (bottom) compounds. Ions were monitored in the full-scan mass spectrum in CI mode. The Cyclodex-B column was used.

Enantiomeric

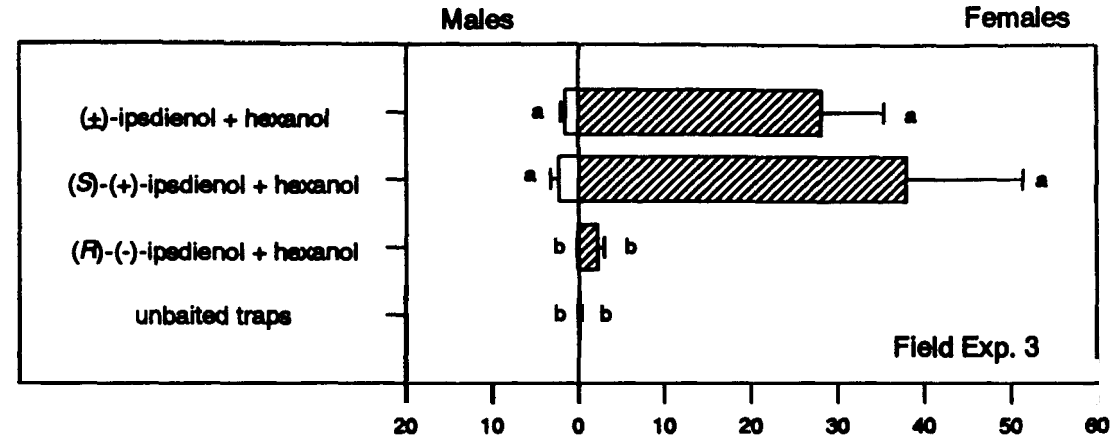


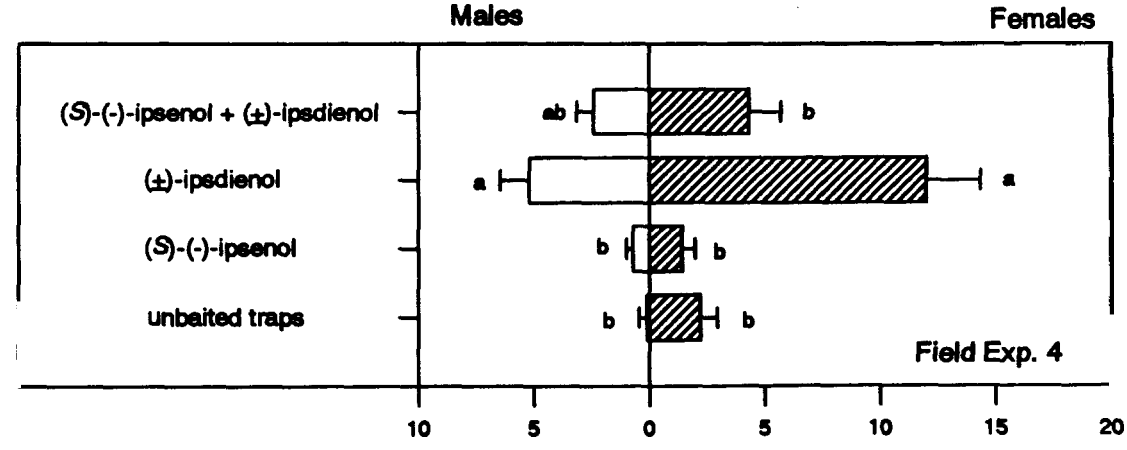
Produced by male *P. knechteli*



RETENTION TIME (min)

Figure 4. Number of *Pityogenes knechteli* caught in multiple funnel traps in fall 1994 (Field Exp. 3) and in summer-fall 1995 (Field Exp. 4). Within an experiment, bars with the same letter for each sex are not significantly different, two-way ANOVA and REGWF test on data transformed by $X' = \log (X + 1)$, $P < 0.05$. In Field Exp. 3, hexanol was released at a rate of approximately 3 mg per 24 h.





Number of Beetles Caught (mean + SE)

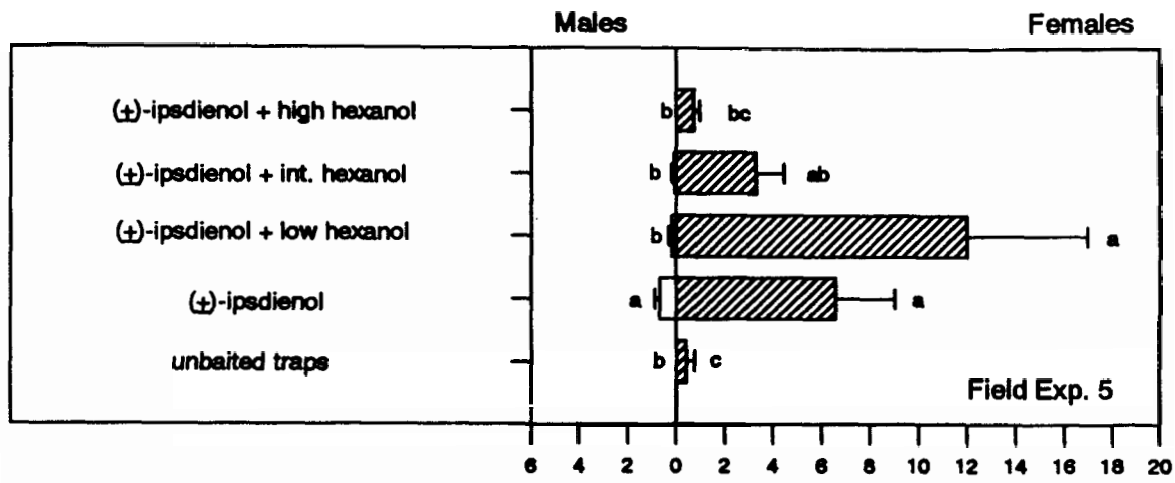
(*S*)-(-)-ipsenol decreased catches to (±)-ipsdienol-baited traps to levels not significantly different from those to the unbaited control traps (Fig. 4).

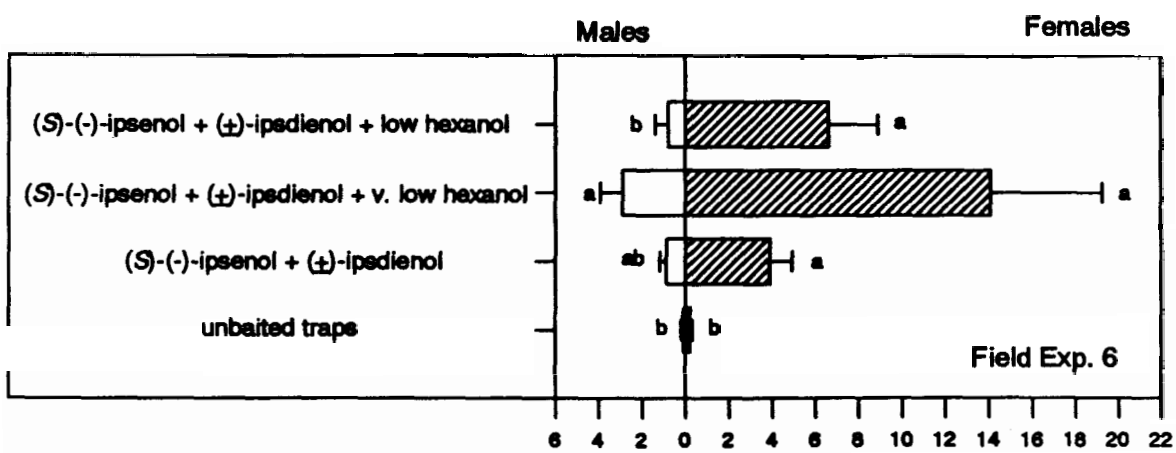
3.3 Role of Hexanol

In Bioassay Exp. 5, a 1:1 combination of (*S*)-(-)-ipsenol and (±)-ipsdienol was significantly attractive compared to the pentane control, for both males and females (Table 2). In this experiment, hexanol, at doses ranging from 0.01 to 1.0 ng, decreased the response to this blend to levels not significantly different from those to the pentane control stimulus. For Bioassay Exp. 6, hexanol at high doses again decreased the response by both sexes to (*S*)-(-)-ipsenol with (±)-ipsdienol. At the highest dose of hexanol the response by females dropped to lower than that to the pentane control (Table 2).

Although traps baited with (±)-ipsdienol and low hexanol in Field Exp. 5 captured numerically more females than traps baited with any other stimulus (Fig. 5), this result was not statistically significant (power, 1-β, > 80 %). As the amount of hexanol released increased, the response declined significantly. Males responded to traps baited with (±)-ipsdienol alone. In Field Exp. 6, a very low release rate of hexanol in combination with ipsenol and ipsdienol was most attractive for both male and female *P. knechteli* but again, not significantly so for females (power, 1-β,> 80 %) (Fig. 5).

Figure 5. Number of *Pityogenes knechteli* caught in multiple funnel traps in fall 1994 (Field Exp. 5) and in summer-fall 1995 (Field Exp. 6). Within an experiment, bars with the same letter for each sex are not significantly different, two-way ANOVA and REGWF test on data transformed by $X' = \log (X + 1)$, $P < 0.05$. Release rates of hexanol were as follows: 0.5, 3.8, 15 and 300 mg per 24 h for very low, low, intermediate and high rates respectively.





Number of Beetles Caught (mean + SE)

3.4 Associated bark beetles

Ips pini were captured in Field Exp. 2, 3 and 5 (Table 3). In Field Exp. 2, inclusion of (±)-ipsenol in the stimulus inhibited the response of *I. pini* to (±)-ipsdienol. In Field Exp. 3, both males and females were attracted in significantly greater numbers to (±)-ipsdienol than to either enantiomer alone or to unbaited traps. Females were slightly attracted to (*R*)-(-)-ipsdienol. Finally, both sexes responded at statistically equivalent levels to all treatments in which (±)-ipsdienol was included in the bait stimulus in Field Exp. 5. At no dose did hexanol cause any change in response in this experiment.

All three associated species were captured in Field Exp. 4 (Table 3). *Ips pini* was attracted by (±)-ipsdienol but was repelled by (*S*)-(-)-ipsenol. *Ips latidens* was attracted by (*S*)-(-)-ipsenol and repelled by (±)-ipsdienol, whereas *I. mexicanus* was attracted to (*S*)-(-)-ipsenol alone, but significantly more so when it was released in combination with (±)-ipsdienol. The combination of the two components was synergistic in attracting *I. mexicanus* females, i.e. the response to the two components together was greater than twice that to (*S*)-(-)-ipsenol alone. *Ips mexicanus* were also captured in Field Exp. 6, in which a low dose of hexanol significantly decreased the response to (±)-ipsdienol plus (*S*)-(-)-ipsenol.

Table 3. Catches of associated bark beetles in Field Exp. 2-6 in multiple funnel traps baited with candidate pheromones for *Pityogenes knechteli.* See Table 1 for release devices and rates.

Field Exp. No.	Species	Stimulus	Number of beetles captured (Mean ± SE) [a]	
			Males	Females
2	*Ips pini*	Unbaited control traps	0.1 ± 0.1a	0.3 ± 0.3 b
		(±)-ipsenol	0.1 ± 0.1a	0 ± 0 b
		(±)-ipsdienol + hexanol	0.3 ± 0.2 a	4.0 ± 1.4 a
		(±)-ipsdienol + hexanol + (±)-ipsenol	0.1 ± 0.1a	0.7 ± 0.4 b
3	*Ips pini*	Unbaited control traps	0 ± 0 b	0 ± 0 c
		(*R*)-(-)-ipsdienol + hexanol	0.2 ± 0.2 b	0.9 ± 0.3 b
		(*S*)-(+)-ipsdienol + hexanol	0.4 ± 0.2 b	0.1 ± 0.1c
		(±)-ipsdienol + hexanol	2.5 ± 0.5 a	7.5 ± 1.8 a
4	*Ips pini*	Unbaited control traps	0.1 ± 0.1 b	0.3 ± 0.2 b
		(*S*)-(-)-ipsenol	0.1 ± 0.1 b	0.2 ± 0.1 b
		(±)-ipsdienol	13.1 ± 3.4 a	45.0 ± 10.9a
		(*S*)-(-)-ipsenol + (±)-ipsdienol	0.2 ± 0.2 b	1.7 ± 0.8 b
	Ips latidens	Unbaited control traps	0.2 ± 0.1 b	0.2 ± 0.1 b
		(*S*)-(-)-ipsenol	9.2 ± 2.7 a	14.2 ± 5.0 a
		(±)-ipsdienol	0.3 ± 0.2 b	0.4 ± 0.2 b
		(*S*)-(-)-ipsenol + (±)-ipsdienol	1.0 ± 0.4 b	0.3 ± 0.2 b

Field Exp. No.	Species	Stimulus	Number of beetles captured (Mean ± SE) [a]	
			Males	Females
4	*Ips mexicanus*	Unbaited control traps	0.1 ± 0.1 c	0.1 ± 0.1 c
		(*S*)-(-)-ipsenol	7.4 ± 1.2 b	10.0 ± 1.7 b
		(±)-ipsdienol	0.4 ± 0.3 c	0.9 ± 0.4 c
		(*S*)-(-)-ipsenol + (±)-ipsdienol	10.9 ± 2.0 a	26.6 ± 3.7 a
5	*Ips pini*	Unbaited control traps	0 ± 0 b	0 ± 0 b
		(±)-ipsdienol	3.3 ± 0.6 a	10.7 ± 2.2 a
		(±)-ipsdienol + low hexanol	3.3 ± 1.4 a	12.8 ± 4.0 a
		(±)-ipsdienol + int. hexanol	2.3 ± 0.6 a	6.2 ± 1.6 a
		(±)-ipsdienol + high hexanol	2.0 ± 0.5 a	5.8 ± 1.1 a
6	*Ips mexicanus*	Unbaited control traps	0 ± 0 c	0.1 ± 0.1 c
		(*S*)-(-)-ipsenol + (±)-ipsdienol	10.1 ± 2.1 a	31.9 ± 6.6 a
		(*S*)-(-)-ipsenol + (±)-ipsdienol + very low hexanol	11.3 ± 2.6 a	28.3 ± 5.7 a
		(*S*)-(-)-ipsenol + (±)-ipsdienol + low hexanol	4.9 ± 1.0 b	14.7 ± 3.9 b

[a] Means within an experiment followed by the same letter are not significantly different, REGWF test, $P < 0.05$.

4. Discussion

The laboratory and field results indicate that ipsdienol is the principal aggregation pheromone component for *P. knechteli.* Males produce (±)-ipsdienol, which is attractive to both males and females. However, only the (*S*)-(+)-enantiomer is used by the beetles, while (*R*)-(-)-ipsdienol is benign (Fig. 4). Ipsdienol is produced by several other members of the tribe Ipini, including several *Ips* species (D.L. Wood 1982; Borden 1985), and two other *Pityogenes* species in Europe (Baader 1989; Francke *et al.* 1995).

Ipsenol is another terpene alcohol commonly found as part of the pheromone components of several members of the tribe Ipini (D.L. Wood 1982; Borden 1985), but it has not been previously reported in the genus *Pityogenes.* (*S*)-(-)-Ipsenol may or may not be produced by male *P. knechteli.* Evidence suggesting that *P. knechteli* produces ipsenol include: (1) presence, in the male extract, of a compound eluting at approximately the same retention time as synthetic ipsenol, and which produces an antennal response in GC-EAD analyses; (2) an antennal response to synthetic ipsenol; (3) presence of ions in the EI spectrum, which are characteristic of ipsenol; and (4) attraction of both males and females to synthetic ipsenol in the laboratory (Table 2). When the extract was analyzed on a chiral column, a compound eluted at the same time as (*S*)-(-)-ipsenol (Fig. 3), but the ion ratio did not match with that of synthetic ipsenol. Therefore absolute confirmation of the presence of ipsenol in the extract cannot be claimed. Most likely, *P. knechteli* produces (*S*)-(-)-ipsenol, but

in a much lower amount than ipsdienol. This would explain why (*S*)-(-)-ipsenol was unattractive in the field when it was tested at an unnaturally high dose and a 1:1 ratio with ipsdienol (Fig. 4, Field Exp. 4).

Hexanol is the other sex-specific compound produced by male *P. knechteli* (Fig. 1). In the cool spring of 1994, when release rates from traps in the shade would have been lower than the rate determined in the laboratory (Table 1), hexanol contributed significantly to the attraction of females when in combination with ipsdienol (Fig. 2). At a very low release rate in the fall of 1995, it also contributed to the attraction of males to a 1:1 blend of ipsenol to ipsdienol (Fig. 5). In all other experiments, however, hexanol was unattractive at low rates and repellent at higher rates. Hexanol was also identified as a male-specific peak in *P. chalcographus* (Francke 1977) and *P. quadridens* (Francke *et al.* 1995). Baader (1989) reported that hexanol reduced response by *P. chalcographus*, although Francke (1977) had observed no apparent effect. It is likely that hexanol is used at very low doses as part of *P. knechteli* pheromone blend to promote aggregation in a newly-attacked host. At high doses it could be employed as an epideictic pheromone (Prokopy 1981) to prevent overpopulation. It would therefore be classed as a multifunctional pheromone, with functions similar to those proposed for several other compounds, *e.g.*, MCH (3-methyl-2-cyclohexen-1-one) in the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins (Rudinsky 1973).

Pityogenes knechteli, I. pini, I. latidens and *I. mexicanus* all co-inhabit the lodgepole pine forest. All four species compete for a scarce phloem resource, and this

competitive pressure has led to a partitioning of the resource which helps them minimize detrimental interactions with each other. This resource partitioning is mediated by several factors, including semiochemical interactions. When the response of all four species to ipsenol and ipsdienol is compared (Fig. 6), it becomes clear that the two terpene alcohol pheromone components are interacting to maintain species-specific communication and to partition host resources.

Pityogenes knechteli and *I. pini* have very similar host requirements, breeding in weakened or downed trees, or at the top of trees attacked by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and the two species often co-exist within the same host, partitioning the phloem resource mostly on the basis of tree diameter (Fig. 7) (Poland and Borden 1994a). *Ips pini* is slightly more aggressive than *P. knechteli*, and will sometimes attack live, standing trees (Furniss and Carolin 1977; A. Savoie, pers. observation). Ipsdienol is the main pheromone component of both species, accounting for the attraction of *P. knechteli* to *I. pini*-infested bolts (Fig. 7, no. 1) (Poland and Borden 1994b). In British Columbia, *I. pini* produces both enantiomers of ipsdienol (Miller *et al.* 1989), but in California, it produces (*R*)-(-)-ipsdienol and is repelled by the antipode, a pheromone component for *I. paraconfusus* (Birch *et al.* 1980a). Thus, one would expect *I. pini* and *P. knechteli* to be incompatible in California unless *P. knechteli* also produces only (*R*)-(-)-ipsdienol in the southern part of its range.

In the field, I have observed co-attack by *I. latidens* and *I. mexicanus* in trees unsuccessfully attacked by the mountain pine beetle, but co-existence of the two

Figure 6. Comparative responses in Field Exp. 4 of *Pityogenes knechteli, Ips pini, I. latidens* and *I. mexicanus* to pheromones produced by male *P. knechteli.* Data normalized for clarity: Similarity of response by sex (Fig. 4, Table 3) justifies pooling of data.

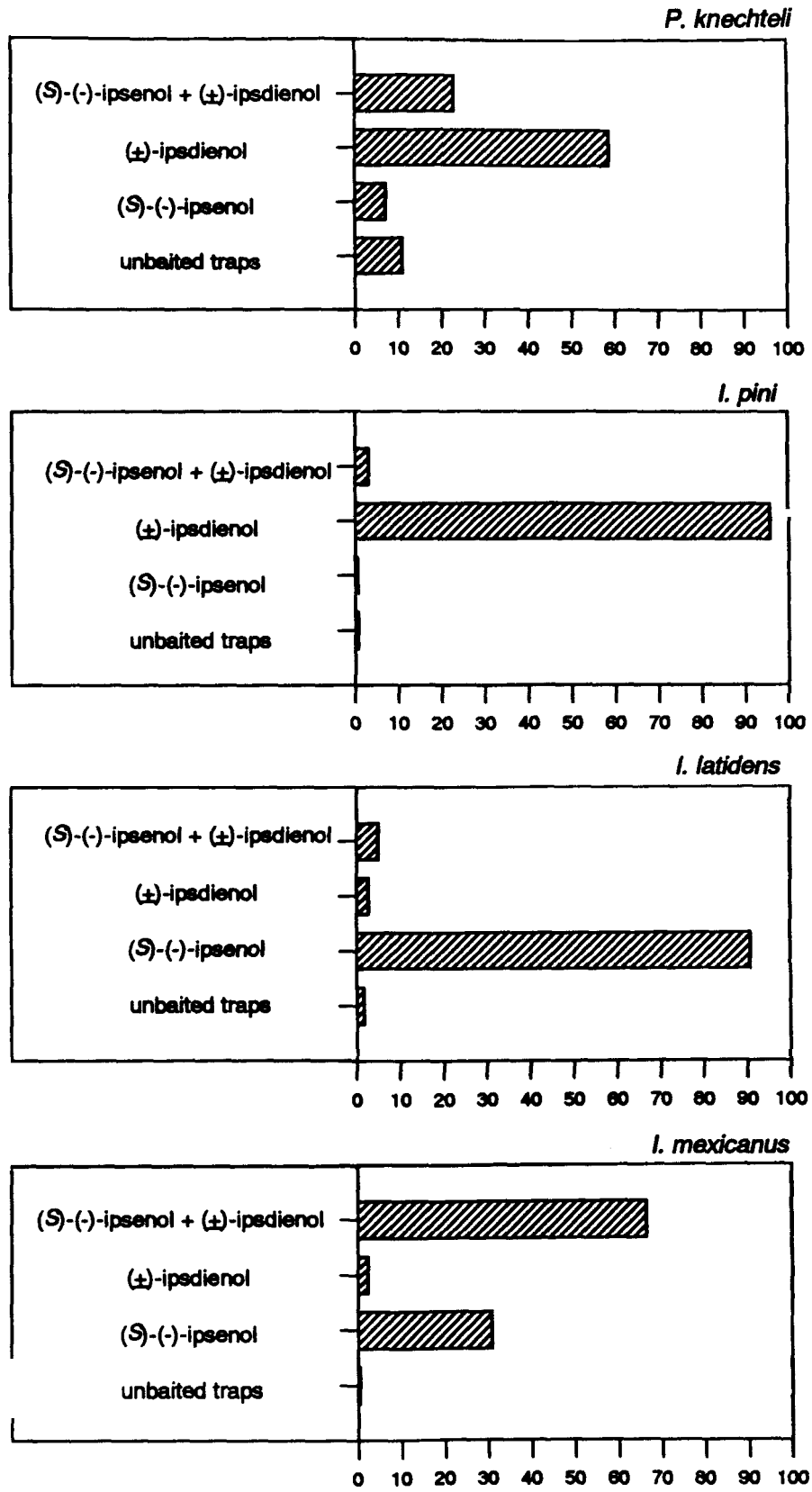
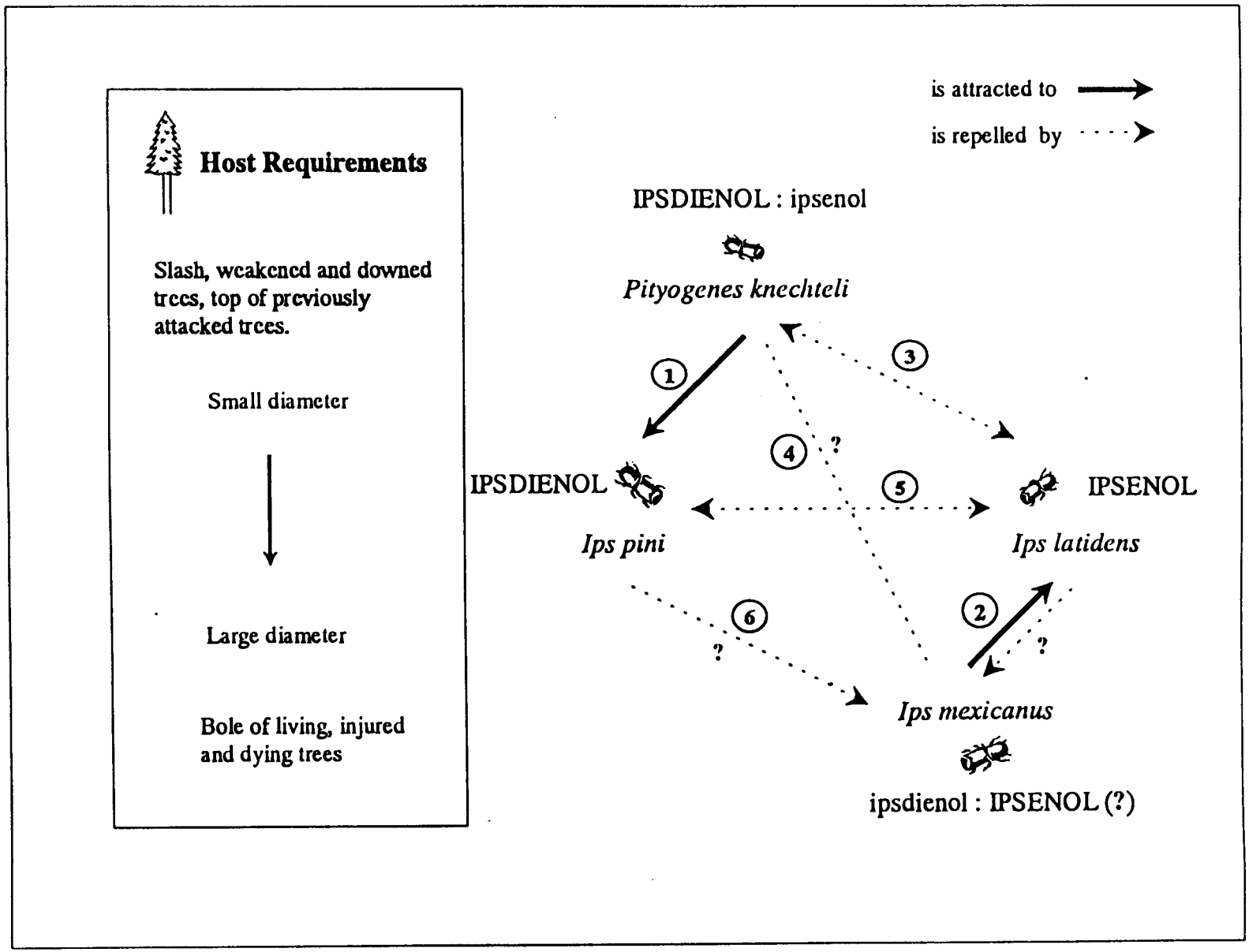

P. knechteli
(S)-(-)-ipsenol + (±)-ipsdienol
(±)-ipsdienol
(S)-(-)-ipsenol
unbaited traps
0 10 20 30 40 50 60 70 80 90 100
I. pini
(S)-(-)-ipsenol + (±)-ipsdienol
(±)-ipsdienol
(S)-(-)-ipsenol
unbaited traps
0 10 20 30 40 50 60 70 80 90 100
I. latidens
(S)-(-)-ipsenol + (±)-ipsdienol
(±)-ipsdienol
(S)-(-)-ipsenol
unbaited traps
0 10 20 30 40 50 60 70 80 90 100
I. mexicanus
(S)-(-)-ipsenol + (±)-ipsdienol
(±)-ipsdienol
(S)-(-)-ipsenol
unbaited traps
0 10 20 30 40 50 60 70 80 90 100
Percent of Total Beetles Captured

Figure 7. Diagram depicting the interactions between *Pityogenes knechteli, Ips pini, I. latidens* and *I. mexicanus*, and their relationship to the relative diameter of host trees or logs usually infested by each species.


Host Requirements
Slash, weakened and downed trees, top of previously attacked trees.
Small diameter
Large diameter
Bole of living, injured and dying trees
is attracted to
is repelled by
IPSDIENOL : ipsenol
Pityogenes knechteli
IPSDIENOL
IPSENOL
Ips pini
Ips latidens
Ips mexicanus
ipsdienol : IPSENOL (?)
1
2
3
4
5
6
?

species within the same host tree does not appear to be as common as for *P. knechteli* and *I. pini*, and the frequency of its occurrence remains to be investigated. *Ips latidens* uses ipsenol as an aggregation pheromone (Miller *et al.* 1991). Although the pheromone produced by *I. mexicanus* has not been investigated, my results suggest that both ipsenol and ipsdienol are part of that species' pheromone system, with ipsenol as the main component (Fig. 6). Co-existence of *I. latidens* and *I. mexicanus* within the same host is surprising if *I. mexicanus* indeed produces ipsdienol, since *I. latidens* is strongly repelled by ipsdienol (Fig. 6) (Miller and Borden 1992). However, this type of interaction is not unusual and has been found in other species. Byers and Wood (1980), for example, found that *D. brevicomis* LeConte and *I. paraconfusus*, which frequently inhabit the same tree, are repelled by each other's pheromone. It is possible that *I. latidens* colonizes the tree first, followed by *I. mexicanus*, which thereby exploits *I. latidens*' pheromone, ipsenol (Fig. 7, no. 2). Testing of this hypothesis awaits definitive information on *I. mexicanus* life history and chemical ecology.

No evidence exists from the literature, or from personal observations, that either *I. latidens* or *I. mexicanus* are ever found breeding in the same tree as *I. pini* or *P. knechteli*. These species likely avoid detrimental competitive interactions by breeding in different hosts. The repellency of both *P. knechteli* and *I. pini* by ipsenol in the field would thus allow them to detect and avoid hosts colonized by their competitors *I. latidens* and *I. mexicanus* (Fig. 7, no. 3-6). Ipsenol is a known inhibitor of *I. pini* attraction, indicating the presence of *I. latidens* (Furniss and Livingston 1979; Borden *et al.* 1992). Similarly, the presence of ipsdienol in the

pheromone blend of *I. pini* and *P. knechteli* would also serve as a synomone, deterring *I. latidens* (Fig. 7, no. 3 and 5). Ipsdienol without ipsenol is unattractive to *I. mexicanus* (Fig. 6), thereby minimizing interactions with *I. pini* (Fig. 7, no. 6). *Ips mexicanus* has different host requirements than *P. knechteli*, attacking mostly the bole of living, injured or dying trees, and thus competitive interactions between the two species might not be pronounced. Furthermore, low levels of hexanol are repellent to *I. mexicanus* (Table 3, Field Exp. 6), suggesting that hexanol could contribute to maintaining species-specific communication between *I. mexicanus* and *P. knechteli*. It appears, then, that ipsdienol, ipsenol and hexanol play an important role for *P. knechteli*, in partitioning host resources with competing species such as *I. latidens* and *I. mexicanus*.

Co-inhabiting the same host, *P. knechteli* and *I. pini* need some way to maintain close-range specificity. I hypothesize that ipsenol is produced in a very low amount and used as a short-range attractant by *P. knechteli*, accounting for its attraction in the laboratory (Table 2), and therefore is a repellent synomone for *I. pini*. Ipsenol is likely produced in a lower amount than ipsdienol (Fig. 1 and 3), making the ratio of ipsenol to ipsdienol critical for optimal response to occur. In the genus *Ips*, for example, maintenance of species specificity is achieved, in part, through variations in the chirality of ipsdienol, and in the relative proportion of ipsenol produced by the males (Vanderwel and Oehlschlager 1987). The attraction of the small southern pine engraver, *Ips avulsus* (Eichhoff), the six-spined ips, *I. calligraphus* (Germar), and the eastern five-spined ips, *I. grandicollis* (Eichhoff), to ipsenol and ipsdienol in the field,

varies with the dose of each semiochemical. A 1:10 ratio of ipsdienol to ipsenol, in combination with *cis*-verbenol, is most attractive to *I. avulsus* and *I. grandicollis*, whereas a 10:1 ratio of the same components is most attractive to *I. calligraphus* (Kohnle *et al.* 1994). Similarly, the European bark beetle *I. amitinus*, which uses (-)-ipsdienol as a pheromone, also produces ipsenol in trace amounts (Francke *et al.* 1980). Because *P. knechteli* produces very low amounts of its pheromone components, the presence of ipsenol in the blend would probably not inhibit *I. pini* from responding to a host colonized by *P. knechteli* but might deter it at close range from superimposing its galleries on those of *P. knechteli*. This would explain the results obtained by Poland and Borden (1994b), wherein *I. pini* showed no attraction to *P. knechteli*-infested logs, but the presence of *P. knechteli* did not decrease its attraction to logs infested by both species.

I suggest that the pheromone produced by *P. knechteli* is not the most important tool used by the beetles in location of host material. During the two years of this study, trap catches have always been extremely low in the spring, whereas a relatively large number of beetles were trapped in late summer-early fall. Thus in the spring, when fresh uninfested host material is abundant and before the emergence of *I. pini*, *P. knechteli* might rely more heavily on host volatiles such as β-phellandrene (Miller and Borden 1990) or ethanol (Klimetzek *et al.* 1986) than on pheromone to locate suitable hosts. Later in the season, however, when fresh uninfested hosts are less abundant and *I. pini* is also seeking the same type of breeding material, *P. knechteli* probably uses mostly ipsdienol as a long-range attractant, and being a less

aggressive species than *I. pini,* exploits the pheromone produced by *I. pini* as an aid in host location. In the southern pine bark beetle guild, *I. grandicollis,* the least aggressive of the five co-habiting species, shows the most cross-attraction to the other species' pheromones, which may facilitate its ability to locate and occupy the wide range of host conditions used by the other species (Smith *et al.* 1990).

It is presumed that the genus *Pityogenes* originated in Eurasia and reached North America rather recently, whereas a North American origin is postulated for the genus *Ips* (S.L. Wood, 1982). In two European studies examining volatiles produced by six species of *Pityogenes,* ipsdienol was found to be produced by two of them, and ipsenol by none (Baader 1989; Francke *et al.* 1995). This information, along with my results, leads me to a final hypothesis about the chemical ecology of *P. knechteli.* Ipsdienol without ipsenol, might have been the main pheromone component of this species, or its ancestor, before its migration to North America. When it reached North America, however, its attraction to ipsdienol would have brought it to trees also colonized by *I. pini.* Due in part to its shorter generation time and its ability to utilize small diameter portions of the host tree, *P. knechteli* would have been able to capitalize on ipsdienol produced by *I. pini* to locate hosts, while avoiding detrimental effects of competition. Ensuing evolution would have reduced the need for a strong attractant in *P. knechteli,* and favored individuals with characteristics that further reduced negative competition with *I. pini.* Thus, *Pityogenes* males that produced ipsenol, made via the same pathway as ipsdienol (Vanderwel and Oehlschlager 1987), and females that responded to ipsenol might have been selected over those that only

produced and responded to ipsdienol, since it would allow them to attract and find a mate more efficiently. This, in turn, would have helped partition resources with *I. pini*, and led to species-specific communication.

5. References

Arn, H., E. Stadler, and S. Rauscher. 1975. The electroantennographic detector - A selective and sensitive tool in gas chromatographic analysis of insect pheromone. *Naturforsch.* **30c**: 722-725.

Atkins, M.D. 1966. Behavioural variation among scolytids in relation to their habitat. *Can. Ent.* **98**: 285-288.

Baader, E.J. Von. 1989. Comparative studies on semiochemicals in ***Pityogenes*** spp. (Col., Scolytidae) and their potential usage in pest management systems. *J. Appl. Ent.* **107**: 1-31.

Benz, G., P. Bovey and P. Junod. 1986. On the specific attraction of the males of the six-toothed spruce bark beetle, *Pityogenes chalcographus* (L.) to a mixture of synthetic pheromones of the eight-toothed spruce bark beetle, *Ips typographus* (L.) (Coleoptera, Scolytidae). *Experientia* **42**: 325-326.

Birch, M.C. 1978. Chemical communication in pine bark beetles. *American Scientist* **66**: 409-419.

Birch, M.C. 1984. Aggregation in bark beetles. *In:* Chemical ecology of insects (W.J. Bell and R.T. Cardé, eds.). Chapman and Hall, New York. pp. 331-353.

Birch, M.C. and D.L. Wood. 1975. Mutual inhibition of the attractant pheromone response by two species of *Ips* (Coleoptera: Scolytidae). *J. Chem. Ecol.* **1**:101-113.

Birch, M.C., D.M. Light, D.L. Wood, L.E. Browne, R.M. Silverstein, B.J. Bergot, G. Ohloff, J.R. West, and J.C. Young. 1980 (a). Pheromonal attraction and allomonal interruption of *Ips pini* in California by the two enantiomers of ipsdienol. *J. Chem. Ecol.* **6**: 703-717.

Birch, M.C., P. Svihra, T.D. Paine and J.C. Miller. 1980 (b). Influence of chemically mediated behavior on host tree colonization by four cohabiting species of bark beetles. *J. Chem. Ecol.* **6**: 395-414.

Borden, J.H. 1974. Aggregation pheromones in the Scolytidae. *In:* Pheromones (M.C. Birch, ed). North-Holland, Amsterdam. pp.135-160.

Borden, J.H. 1977. Behavioral response of Coleoptera to pheromones, allomones, and kairomones. *In*: Chemical Control of Insect Behavior: Theory and Application (H.H. Shorey and J.J. McKelvey, Jr., eds). John Wiley & Sons, New York. pp.169-198.

Borden, J.H. 1985. Aggregation pheromones. *In*: Comprehensive insect physiology, biochemistry and pharmacology, Vol. 9 (G.A. Kerkut and L.I. Gilbert, eds.). Pergamon Press, Oxford. pp. 257-285.

Borden, J.H. 1996. Disruption of semiochemical-mediated aggregation in bark beetles. *In*: Insect pheromones: new directions (R.T. Cardé and A.K. Minks, eds.). Chapman and Hall, N.Y. (In Press).

Borden, J.H., D.R. Devlin, and D.R. Miller. 1992. Synomones of two sympatric species deter attack by the pine engraver, *Ips pini* (Coleoptera: Scolytidae). *Can. J. For. Res.* **22**: 381-387.

Bright, D.E. Jr. 1976. The insects and arachnids of Canada Part 2: The bark beetles of Canada and Alaska. Agriculture Canada Publication 1576, Ottawa. 241 pp.

Byers, J.A. 1989. Chemical ecology of bark beetles. *Experientia* **45**: 271-283.

Byers, J.A. 1993. Avoidance of competition by spruce bark beetle, *Ips typographus* and *Pityogenes chalcographus*. *Experientia* **49**: 272-275.

Byers J.A. and D.L. Wood. 1980. Interspecific inhibition of the response of the response of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus*, to their pheromones in the field. *J. Chem. Ecol.* **6**: 149-164.

Day, R.W. and G.P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. *Ecol. Monogr.* **59**: 433-463.

Francke, W. 1977. 2-Ethyl-1,6-dioxaspiro[4.4]nonane, Principal aggregation pheromone of *Pityogenes chalcographus* (L.) *Naturwissenschaften* **64**: 590-591.

Francke, W., P. Sauerwein, J.P. Vité, and D. Klimetzek. 1980. The pheromone bouquet of *Ips amitinus*. *Naturwissenschaften* **67**: 147-148

Francke, W., J. Bartels, H. Meyer, F. Schroder, U. Kohnle, E. Baader and J.P. Vité. 1995. Semiochemicals from bark beetles: New results, remarks, and reflections. *J. Chem. Ecol.* **21**: 1043-1063.

Furniss, M.M. and R.L. Livingston. 1979. Inhibition by ipsenol of pine engraver attraction in northern Idhao. *Environ. Ent.* **8**: 369-372.

Furniss, R.L. and V.M. Carolin. 1977. Western forest insects. USDA Forest Service, Misc. Public. 1339. 654pp.

Gries, G. 1995. Prospects of new semiochemicals and technologies. *In*: Application of semiochemicals for management of bark beetle infestations - Proceedings of an informal conference. USDA - Forest Service, Intermountain Research Station, General Technical Report INT-GTR-318. pp. 44-47.

Kelsey, R.G. 1993. Ethanol synthesis in Douglas-fir logs felled in November, January, and March and its relationship to ambrosia beetle attack. *Can. J. For. Res.* **24**: 2096-2104.

Kelsey, R.G. 1994. Ethanol and ambrosia beetles in Douglas fir logs with and without branches. *J. Chem. Ecol.* **20**: 3307-3319.

Klimetzek, D., J. Köhler, J.P. Vité, and U. Kohnle. 1986. Dosage response to ethanol mediates host selection by 'secondary' bark beetles. *Naturwissenschaften* **73**: 270-272.

Kohnle, U., J.P. Vité, H. Meyer, and W. Francke. 1994. Response of four American engraver bark beetles, *Ips* spp. (Col., Scolytidae), to synthetic racemates of chiral pheromones. *J. Appl. Ent.* **117**: 451-456.

Lindgren, B.S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). *Can. Ent.* **115**:299-302.

Miller, D.R. 1991. Reproductive and ecological isolation: Community structure in the use of semiochemicals by pine bark beetles (Coleoptera: Scolytidae). Ph. D. thesis, Simon fraser University, Burnaby, B.C., 166 pp.

Miller, D.R. and J.H. Borden. 1985. Life history and biology of *Ips latidens* (LeConte) (Coleoptera: Scolytidae). *Can. Ent.* **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. and J.H. Borden. 1992. (S)-(+)-ipsdienol: Interspecific inhibition of *Ips latidens* (Leconte) by *Ips pini* (Say) (Coleoptera: Scolytidae). *J. Chem. Ecol.* **18**: 1577-1583.

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, G.G.S. King, and K.N. Slessor. 1991. Ipsenol: an aggregation pheromone for *Ips latidens* (LeConte) (Coleoptera: Scolytidae). *J. Chem. Ecol.* **17**: 1517-1527.

Miller, D.R., J.H. Borden, and K.N. Slessor. 1996. Enantiospecific pheromone production and response profiles for populations of the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae), in British Columbia. *J. Chem. Ecol.* (submitted).

Moeck, H.A. 1981. Ethanol induces attack on trees by spruce beetles, *Dendroctonus rufipennis* (Coleoptera: Scolytidae). *Can. Ent.* **113**: 939-942.

Nordlund, D.A. 1981. Semiochemicals: A review of the terminology. *In*: Semiochemicals: Their role in pest control. (D.A. Nordlund, R.L. Jones and W.J. Lewis, eds.). John Wiley & Sons, New York. pp. 13-28

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.

Poland, T.M. 1993. Interspecific interactions between *Ips pini* Say and *Pityogenes knechteli* Swaine in lodgepole pine. M.P.M. thesis, Simon Fraser University, Burnaby, B.C., 50 pp.

Poland, T.M. and J.H. Borden. 1994a. Attack dynamics of *Ips pini* (Say) and *Pityogenes knechteli* (Swaine) (Col., Scolytidae) in windthrown lodgepole pine trees. *J. Appl. Ent.* **117**: 434-443.

Poland, T.M. and J.H. Borden. 1994b. Semiochemical-based communication in interspecific interactions between *Ips pini* (Say) and *Pityogenes knechteli* Swaine) (Coleoptera: Scolytidae) in lodgepole pine. *Can. Ent.* **126**: 269-276.

Prokopy, R.J. 1981. Epideictic pheromones influencing spacing patterns of phytophagous insects. *In*: Semiochemicals: Their role in pest control. (D.A. Nordlund, R.L. Jones, and W.J. Lewis, eds.). John Wiley and Sons, New York. pp. 181-213.

Reid, R.W. 1955. The bark beetle complex associated with lodgepole pine slash in Alberta. *Can. Ent.* **87**:311-323.

Rudinsky, J.A. 1962. Ecology of Scolytidae. *Ann. Rev. Ent.* 7:327-348.

Rudinsky, J.A. 1973. Multiple functions of the Douglas-fir beetle pheromone 3-methyl-2-cyclohexen-1-one. *Environ. Ent.* **2**: 579-585.

SAS Institute. 1994. SAS System for Personal Computers, Release 6.10. SAS Institute Inc., Carey, North Carolina.

Seybold, S.J., S. A. Teale, D.L. Wood, A. Zhang, F.X. Webster, K.Q. Lindhal Jr., and I. Kubo. 1992. The role of lanierone in the chemical ecology of *Ips pini* (Coleoptera: Scolytidae) in California. *J. Chem. Ecol.* **18**: 2305-2329.

Seybold, S.J., T. Ohtsuka, D.L. Wood, and I. Kubo. 1995. Enantiomeric composition of ipsdienol: a chemotaxonomic character for North American populations of *Ips spp.* in the *pini* subgeneric group (Coleoptera: Scolytidae). *J. Chem. Ecol.* **21**: 995-1016.

Smith, M.T., T.L. Payne, and M.C. Birch. 1990. Olfactory-based behavioral interactions among five species in the southern pine bark beetle group. *J. Chem. Ecol.* **16**: 3317-3331.

Stock, A.J. 1981. The western balsam bark beetle, *Dryocetes confusus* Swaine: Secondary attraction and biological notes. M. Sc. Thesis, Simon Fraser University, Burnaby, B.C.

Stock, A.J. and J.H. Borden. 1983. Secondary attraction in the western balsam bark beetle, *Dryocetes confusus* (Coleoptera: Scolytidae). *Can. Ent.* **115**: 539-550.

Svihra, P., T.D. Paine, and M.C. Birch. 1980. Interspecific olfactory communications in southern pine beetles. *Naturwissenschaften* **67**: 518-520.

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.

Vanderwel, D. and A.C. Oehlschlager. 1987. Biosynthesis of pheromones and endocrine regulation of pheromone production in Coleoptera. *In*: Pheromone Biochemistry (G.D. Prestwich and G.J. Blomquist, eds.). Academic Press. pp. 175-215.

Wood, D.L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Ann. Rev. Ent.* **27**: 411-446.

Wood, D.L. and R.W. Bushing. 1963. The olfactory response of *Ips confusus* (LeConte) (Coleoptera: Scolytidae) to secondary attraction in the laboratory. *Can. Ent.* **95**: 1066-1078.

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae). *Mem. Great Basin Natur.* **6**:1359 pp.

Zar, J.H. 1984. Biostatistical Analysis. Prentice-Hall Inc., Englewood Cliffs, N.J.

Zuber, M. Von and G. Benz. 1992. Investigations on the temporary sequence of the flight activities of *Ips typographus* (L.) and *Pityogenes chalcographus* (L.) (Col., Scolytidae) by means of the commercial pheromone preparations Pheroprax and Chalcoprax. *J. Appl. Ent.* **113**: 430-436.