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Dynamics of pheromone production and communication in the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae)

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Summary. The mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and the pine engraver, *Ips pini* (Say), often co-exist in lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann. Intra- and interspecific semiochemical communication occurs in both species and their complete semiochemical repertoire and precise dynamics of pheromone production have not been elucidated. Porapak-Q extracts of captured volatiles from beetles of each 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 (seven compounds common to both species, six present in *D. ponderosae* and four present in *I. pini*) that excited the antennae of either or both species. Seven compounds for *D. ponderosae* and nine for *I. pini* had not been assessed for behavioural activity. In field trapping experiments, 2-phenylethanol 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-phenylethanol in emergent *D. ponderosae* suggests that these compounds may inhibit aggregation and induce dispersal following emergence. Termination of aggregation in *D. ponderosae* appears to depend on the production of frontalin in combination with changes in the relative ratios of verbenone, *exo*-brevicomin, *trans*-verbenol and 2-phenylethanol. In *I. pini*, the cessation of ipsdienol production by males is probably the main factor in terminating aggregation.

Key words. Aggregation – antiaggregation – synomones – Coleoptera – Scolytidae – *Dendroctonus ponderosae* – *Ips pini*

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Introduction

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins, is the most lethal natural agent of lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann, in western North America (Furniss & Carolin 1977; Van Sickle 1989). Pioneer females initiate attack and 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 *et al.* 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 & Vité 1970). The phloem resource on the tree is limited and overcrowding results in intraspecific competition which is detrimental to the survival of brood. The optimum attack density is 62 galleries per m^2 (Raffa & Berryman 1983). As this density is approached, aggregation is terminated by the production of high concentrations of the multifunctional pheromones *exo*-brevicomin and frontalin by males (Ryker & Rudinsky 1982; Ryker & Libbey 1982; Borden *et al.* 1987) and verbenone by intestinal- and gallery-inhabiting microbes in both sexes (Leufvén *et al.* 1984; Hunt & Borden 1989, 1990). This signals the unavailability of phloem in the attacked tree and causes incoming conspecifics to switch the attack to trees nearby (Rudinsky *et al.* 1974a; Geiszler & Gara 1978; Geiszler *et al.* 1980; Ryker & Libbey 1982; Ryker & Yandell 1983).

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 & Benjamin 1969; Schmitz 1972). It commonly infests the upper and lower boles of lodgepole pine attacked by *D. ponderosae* (Hopping 1961; Furniss & Carolin 1977; Amman & Safranyik 1985; Safranyik & Linton 1991). On initiating nuptial chambers, males produce the aggregation pheromone ipsdienol, which attracts both males and females to the site (Swaby &

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). As soon as females are acquired, male attraction is rapidly terminated (Reid & Roitberg 1994). The mechanism of termination of aggregation in *I. pini* has not been elucidated and females are not known to produce any kind of pheromone (Borden *et al.* 1991; Lissemore 1997).

Ips pini frequently inhabits trees attacked by *D. ponderosae*, resulting in exploitative competition for food and space between the two species (Hopping 1961; Amman & Safranyik 1985; Rankin & Borden 1991). 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 & Knight 1972; Rankin & Borden 1991). However, *D. ponderosae* larvae are larger than those of *I. pini* and may have an advantage in aggressive encounters. 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 & Borden 1991). Therefore, mutual semiochemical-based inhibition would be an adaptive mechanism to avoid competition for the same resource (Borden 1975; Byers 1989) and minimize 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 & Keen 1960; Stark & Borden 1965; Ashraf & Berryman 1969; McCambridge & Knight 1972; Berryman 1973; Light & Birch 1979; Paine *et al.* 1981; Borden *et al.* 1991; Rankin & Borden 1991; Devlin & Borden 1994; Safranyik *et al.* 1996; Poland & Borden 1998a,b). Hunt & Borden (1988) 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, myrcene in combination with *trans*-verbenol and *exo*-brevicomin, both aggregation pheromones of *D. ponderosae*, decreased the response of *I. pini* to traps baited with ipsdienol (Hunt & Borden 1988). In another study, there was a weakly significant decrease in attraction of *I. pini* with an increasing dose of *exo*-brevicomin; and a much stronger relationship for a blend of *cis*- and *trans*-verbenol (Miller 1990). Verbenone was also highly repellent to *I. pini*, and inhibited attack on ipsdienol-baited logs (Borden *et al.* 1991; Devlin & Borden 1994). The complete semiochemical profiles of both *D. ponderosae* and *I. pini* and their full potential interactions have not been studied in detail.

Our 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 *et al.* 1992); 2) to test antenally-active compounds of unknown or uncertain behavioural activity in the field and to determine their potential role in intra- and interspecific communication; and 3) to elucidate the dynamics of semiochemical production by qualitative and quantitative analyses of volatiles at various phases of attack.

Materials and methods

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 trees were felled and baited with ipsdienol to induce attack by *I. pini*. Infested logs were cut into bolts 0.5 m long, the cut surfaces were sealed with paraffin and the bolts were stored at 4°C. Bolts from uninfested trees were similarly 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).

Experimental treatments

Beetles were classified into five treatment groups: 1) freshly emerged males and 2) females, 3) female *D. ponderosae* and male *I. pini*, pioneer sexes, 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 one to three females after 24 h of boring. Excisions were made after 48 h of attack in treatment 3 for both species and after 96 h in treatments 4 and 5. Galleries were dissected using a pocketknife and beetles removed from the bark by gently prodding them with a plastic toothpick.

Aerations of grouped beetles in glass tubes

Fifty beetles from each of the above attack phases were aerated as a pooled group in Pyrex® tube aeration chambers (1.2 cm OD, 18 cm long), a modified version of Rudinsky's (1974) apparatus (Gries *et al.* 1992). Charcoal filtered air was drawn at ca. 1.5 L per min through the tube until the last beetle died. 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.

Analysis of volatiles

Volatiles from tube aerations were subjected to gas chromatographic-electroantennographic detection (GC-EAD) analyses (Arn *et al.* 1975) using the antennae of males and females of both species and a Hewlett Packard 5890 gas chromatograph equipped with a fused silica column (DB-5, 30 m × 0.25 mm ID, J&W Scientific, Folsom, CA 95630). 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. 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 240°C. The temperatures of the injection and detector ports were 250°C and 260°C, respectively. Calculation of retention indices (van den Dool & Kratz 1963), co-chromatography with authentic synthetic standards and spectral comparisons were used to identify antennally-active compounds. All such compounds were then quantified using 1 ng/µl of n-decanol as the internal standard on a Hewlett Packard 5890A gas chromatograph equipped with a fused silica column, DB-5 for *D. ponderosae* and DB-23 (30 m × 0.32 mm ID) for *I. pini*, with settings as above except for the final temperature of the DB-23 column which was 200°C.

To determine the enantiomeric composition of chiral compounds, samples containing sufficient amounts of target compounds were analysed on a cyclodex B column, 30 m × 0.25 mm ID (J&W Scientific Folson CA 95630-4714) with an initial temperature of 60°C, increased at 10°C/min to 115°C for 16 min, then increased at 5°C/min to 120°C, for 20 min for *I. pini*. For *D. ponderosae*, the initial temperature was 60°C, increased at 5°C/min to 80°C, for 10°C min, then increased at 10°C/min to 100°C for 8 min, and further increased at 10°C/min to 115°C. The samples were also run on a γ-cyclodextrin-trifluoroacetyl, 40 m × 0.25 m ID (Advanced Separation Technologies Inc. (ASTEC), Whippany, NJ 07981). A 5 m DB-5 column (0.32 mm ID) was used as a precolumn. The initial temperature was 60°C, increased at 2°C/min to 100°C for 20 min for both species.

Aerations of lodgepole pine

To determine if volatiles from beetles were also produced by host trees, aerations 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 2–3 cm thick discs and aerated as above in 10 L plastic chambers 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 5 cm of Porapak-Q. The traps were eluted with 150 ml of distilled pentane. The extracts were concentrated to 4 ml by distillation of solvent through a 30 cm long Dufton column and analysed on a Hewlett Packard 5890A gas chromatograph with settings as above. The amount of nonanal was quantified using synthetic nonanal as an external standard.

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 7 days after one or two females were acquired. The beetles were 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.

For comparison with bolts attacked in the laboratory, a lodgepole pine tree was baited with *trans*-verbenol and *exo*-brevicomin at 1500 h 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.

Field trapping experiments

Compounds that elicited antennal responses and for which bioactivity was unknown or uncertain, 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 $\geq$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*-brevicomin for *D. ponderosae* and ipsdienol for *I. pini*, to detect any potential enhancement or inhibition of attraction. The source, purity, chiral composition, release device and rate for each compound are given in Table 1. A separate GC-EAD analysis was done as above on both sexes of *I. pini* to determine which enantiomers of *exo*- and *endo*-brevicomin could be detected. Captured insects from all experiments were frozen in plastic bags until sexed (Lyon 1958; Lanier and Cameron 1969) and counted.

Statistical analyses

Data from the individual beetle extractions did not conform to the assumptions of normality and homoscedasticity demanded by parametric statistics. Therefore, Wilcoxon rank sums and Kruskal-Wallis tests were used for two and three treatments respectively, followed by non-parametric multiple comparisons for data with three treatments (Zar 1984; Day & Quinn 1989; SAS Institute Inc. 1990), to determine differences between mean amounts of compounds across two or three attack phases for both species. For field trapping experiments, 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 1984; Day & Quinn 1989; SAS Institute Inc. 1990). In all cases $\alpha = 0.05$.

Results

Production of volatiles by grouped beetles in glass tubes

Twelve compounds found in the volatiles of *D. ponderosae* elicited an antennal response from either or both species (Table 2, Fig. 1). *exo*-Brevicomin and *endo*-brevicomin 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-Phenylethanol 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.

Eleven compounds found in the volatiles of *I. pini* excited the antennae of either or both species (Table 2, Fig. 2). 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

Table 1 Compounds tested for behavioral activity in the field against *D. ponderosae* and *I. pini*

Chemical	Source[a]	Chemical purity (%)	Enantiomeric composition[b]	Release device	Target species[c]	Release rate (mg/24 h)[d]
ATTRACTANT BAITS						
myrcene	Phero tech	93	NA	20 ml low density polyethylene bottle	MPB	95
exo-brevicomin	Phero tech	99	(±)	flexlure	MPB	0.3
trans-verbenol	Phero tech	75	18%(+) : 82%(−)	bubble cap	MPB	1.5
ipsdienol	Phero tech	97	(±)	bubble cap	PE	0.11
TEST COMPOUNDS						
nonanal	Aldrich	95	NA	1.5 ml polypropylene microtube with 4 pores	PE	6.8
2-phenylethanol	Sigma	98	NA	five 1.5 ml polypropylene microtube with 4 pores	MPB, PE	4.2
acetophenone	Aldrich	99	NA	1.5 ml polypropylene microtube with 4 pores	MPB, PE	6.7
borneol	Sigma	88	11%(+) : 89%(−)	three 20 ml low density polyethylene bottle with 16 pin pricks	MPB, PE	4.1
p-cymene	Aldrich	99	NA	1.5 ml polypropylene microtube with 1 pore	MPB	6.9
endo-brevicomin	Phero Tech	93	(±)	two 250 µl polyethylene microtube with 8 pin pricks	PE	6.6
exo-brevicomin	Phero Tech	99	(±)	250 µl polyethylene microtube with 8 pin pricks	PE	3.1
cis/*trans* limonene oxide	Aldrich	97	(±)	1.5 ml polypropylene microtube with 2 pores	MPB, PE	5.2
myrtenol	Aldrich	95	3%(+) : 97%(−)	four 1.5 ml polypropylene microtube with 4 pores	MPB, PE	5.1
1-phenylethanol	Aldrich	98	NA	two 1.5 ml polypropylene microtube with 4 pores	MPB, PE	5.9

[a] Phero Tech Inc., 7572, Progress Way, Delta, BC, Canada V4G 1E9; Aldrich Chemical Company Inc., Milwaukee, WI 53233, USA; Sigma Chemical Company, St. Louis, MO 63178, USA
[b] NA = not applicable
[c] MPB = mountain pine beetle, *D. ponderosae*; PE = pine engraver, *I. pini*
[d] Release rates determined at 25°C in the laboratory

amount 72 h after 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 was released in large amounts in freshly-emerged beetles of both sexes. *trans*-Verbenol and verbenone were present in greater amounts in the volatiles of freshly-emerged beetles than in the other phases of attack. Nonanal was detected both in beetles that were in the log alone and in those that had acquired mates. 2-Phenylethanol produced 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 were also detected in paired males in relatively high amounts. Borneol was present in high amounts in the volatiles of paired males. Myrtenol was detected in freshly-emerged beetles, with females containing trace amounts.

Table 3 depicts the enantiomeric compositions of the chiral compounds detected in both species.

GC-MS analyses of volatiles from uninfested lodgepole pine revealed the presence of nonanal in all four samples captured at rates of 0.5, 0.8, 1.7 and 1.8 ng/h/g tissue for trees from Sunday Summit, Whipsaw Creek, Lytton and Whipsaw Creek respectively. These rates represented 0.03–0.06% of the total volatile release rate.

Individual beetle extractions

Results from the individual beetle extractions for *D. ponderosae* (Fig. 3) reflect those of 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-Phenylethanol 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.

Paired *D. ponderosae* females excised from the field-attacked tree had significantly more *trans*-verbenol and verbenone than females without mates (Table 4). Females had trace amounts of *exo*-brevicomin, *endo*-brevicomin and frontalin. There was no difference in

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

Source species and compound	Sex in which compound was detected				Antennal response (male or female)	
	D. ponderosae		*I. pini*		*D. ponderosae*	*I. pini*
	males	females	males	females		
Both species						
cis-verbenol	+	+	+	+	+	+
trans-verbenol		+	+	+	+	+
verbenone	+	+	+	+	+	+
nonanal	+	+	+	+	+	+
2-phenylethanol	+	+	+	+	+	+
acetophenone	+	+	+	+	+	+
borneol	+	+	+	+	+	+
D. ponderosae						
exo-brevicomin	+				+	+
endo-brevicomin	+				+	+
frontalin	+				+	
p-cymene	+	+			+	+
cis-limonene oxide	+	+			+	+
trans-limonene oxide	+	+			+	+
I. pini						
ipsdienol			+	+	+	+
lanierone			+			+
1-phenylethanol			+	+	+	+
myrtenol			+	+	+	+

the amounts of any of the other compounds between single and paired females.

Quantitative analyses of compounds detected in individual *I. pini* (Fig. 4) 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-Phenylethanol 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.

Field trapping experiments

Of the seven compounds tested against *D. ponderosae*, only 2-phenylethanol was behaviourally active (Fig. 5). It significantly reduced the attraction of both sexes to the aggregation 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 (Fig. 6). *D. ponderosae* produces predominantly (+)- *exo*- and (+)- *endo*-brevicomin. Racemic blends were tested in the field against *I. pini*. GC-EAD analyses of (+) and (−) *exo*- and *endo*-brevicomin against male and female *I. pini* disclosed that only the (+) enantiomers elicited antennal responses. Therefore the antipodes would have had no behavioural activity.

Despite the fact that 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.

Discussion

Volatile production by D. ponderosae

Data from the tube aerations and individual extractions were not entirely consistent (Figs 1, 3). The lack of *trans*-verbenol and verbenone in individual extractions of emergent beetles is consistent with previous studies (Pitman & Vité 1969; Hughes 1973a,b; Gries *et al.* 1990b). Production of *trans*-verbenol from the volatiles of pooled emerged beetles in aeration tubes was possibly due to spontaneous or microbial oxidation of α-pinene in the guts of dying beetles (Hunt *et al.* 1989; Hunt & Borden 1990). Normally, emerged beetles may avoid being attractive by metabolising *trans*-verbenol into verbenene, *p*-mentha-1,5,8-triene or cymene (Gries *et al.* 1990b). The detection of *trans*-verbenol in high amounts in both aerations and extractions of fed females is consistent with Pierce *et al.* (1987).

The detection of *exo*- brevicomin in male *D. ponderosae* at both phases of attack is consistent with its primary role as an aggregation pheromone. In contrast, the presence of frontalin only in males paired with

females reaffirms its probable major role as an antiaggregant (Borden *et al.* 1987). Trace amounts of brevicomin and frontalin were seen in females from the field-attacked tree (Table 4). 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. In nature, females may use trace amounts of frontalin and *exo*-brevicomin to synergise the aggregation potential of *trans*-verbenol.

Verbenone has antiaggregative properties for a number of scolytid beetles (Shore *et al.* 1992; Borden

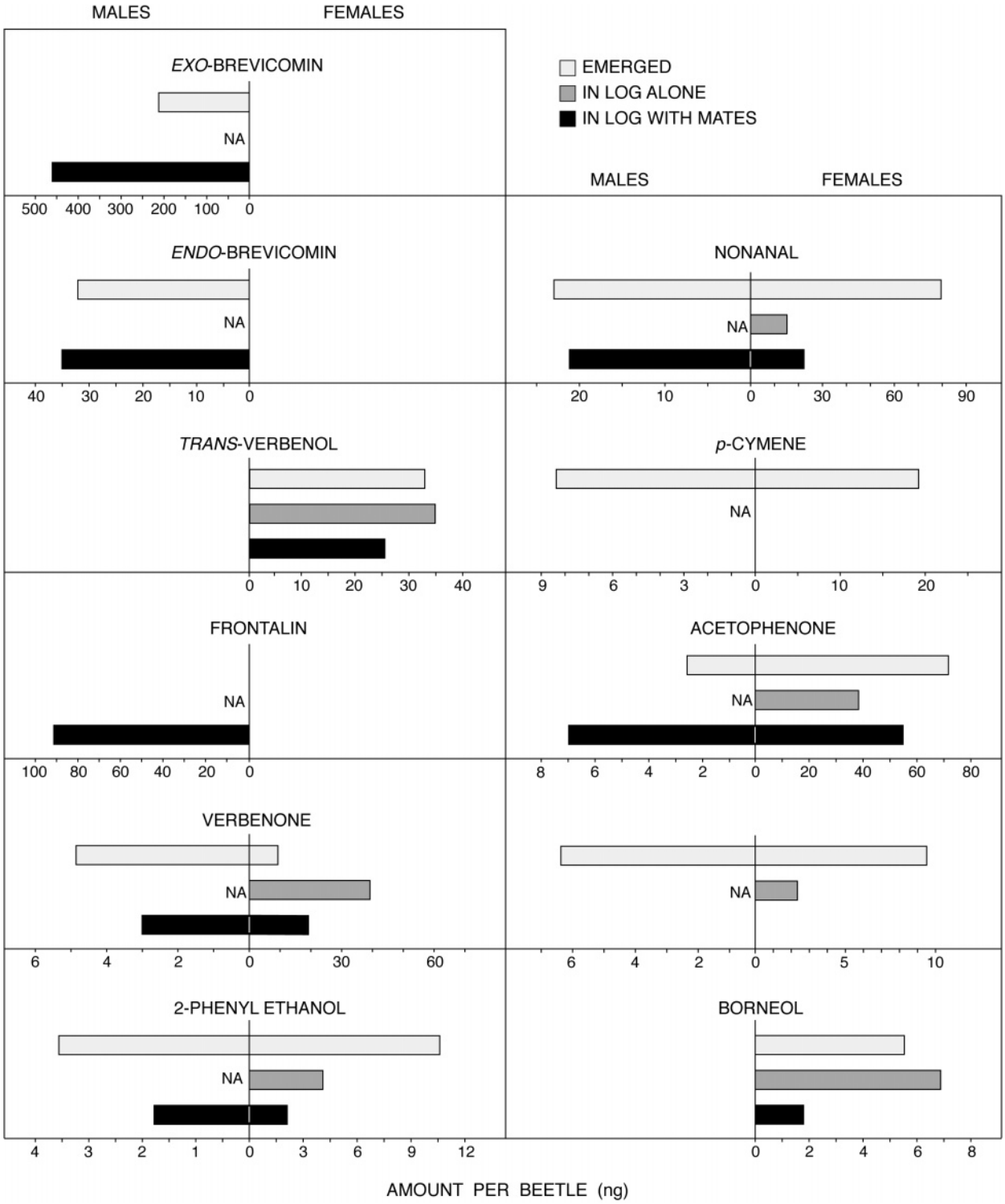

Fig. 1 Amounts of antennally-active compounds per beetle as determined by gas chromatographic analyses of pooled volatiles from 50 male and 50 female *D. ponderosae* aerated at two and three attack phases, respectively. Note variable scales for amounts per beetle. NA = Not Applicable

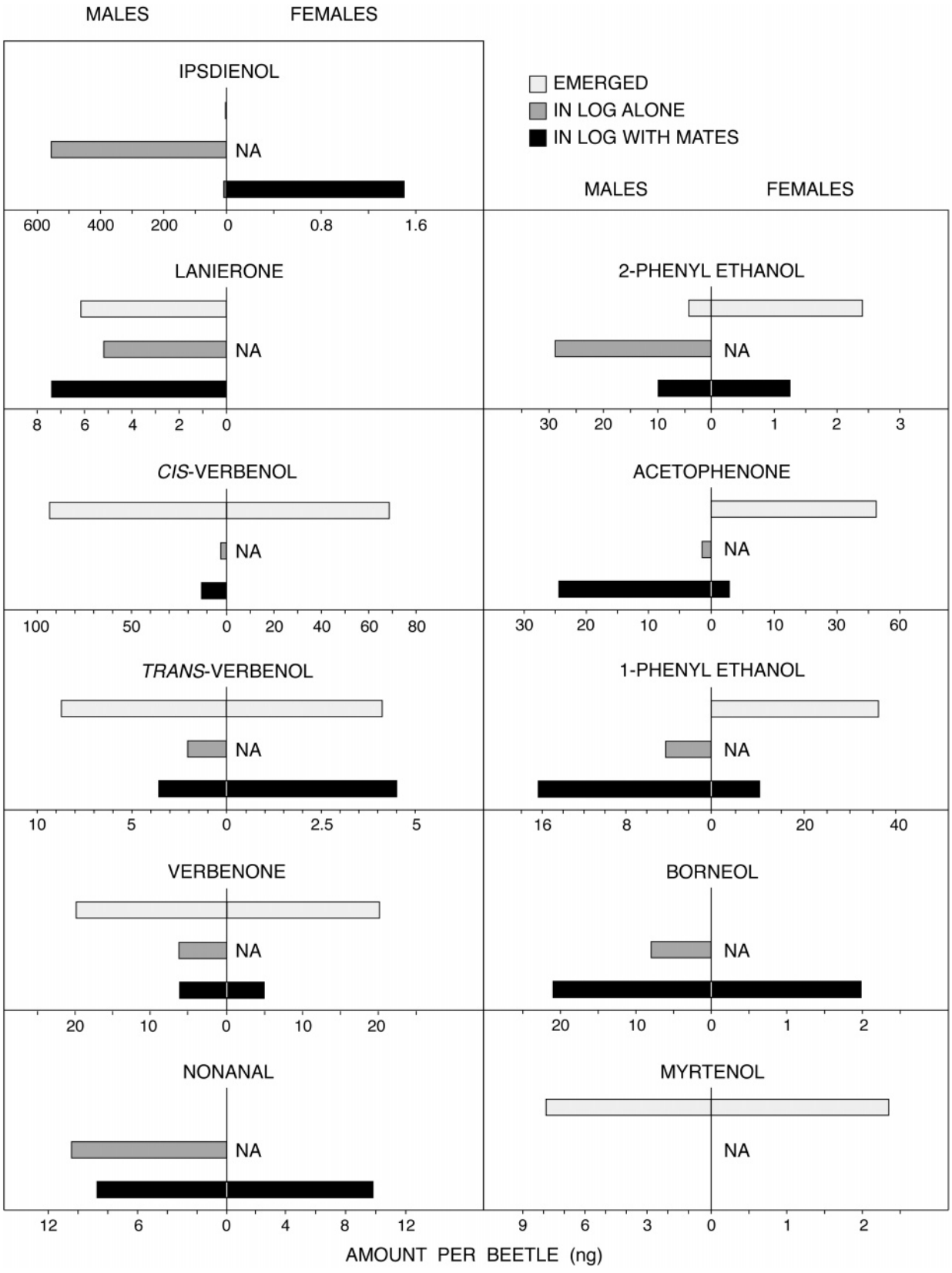

Fig. 2 Amounts of antennally-active compounds per beetle as determined by gas chromatographic analyses of pooled volatiles from 50 male and 50 female *I. pini* aerated at three and two attack phases, respectively. Note variable scales for amounts per beetle. NA = Not Applicable

Table 3 Enantiomeric composition of antennally active compounds in the volatiles obtained from group aerations

Species	Compound	Attack phase(s) for which enantiomeric composition was determined	%(+)[a]	%(−)[a]
D. ponderosae	*exo*-brevicomin	emerged males	98	2
		paired males	98	2
	endo-brevicomin	emerged males	93	7
		paired males	90	10
	cis-verbenol		ND	ND
	trans-verbenol	emerged females	11	89
		females in log alone	11	89
	frontalin	paired males	7	93
	cis/*trans*-limonene oxide	emerged males	65 (+) *trans*-	35 (mixture of (−) *trans*-, (+) *cis*- and (−) *cis*-)
	verbenone	emerged females	20	80
	borneol	emerged females	trace	≤100
		females in log alone	trace	≤100
I. pini	ipsdienol	males in log alone	57	43
	cis-verbenol	emerged males	2	98
		emerged females	ND	100
	trans-verbenol	emerged males	ND	100
		emerged females	ND	100
	verbenone	emerged males	ND	100
		emerged females	ND	100
	borneol	males with females	~25[b]	~75[b]
	myrtenol	emerged males	ND	100

[a] ND = not detectable (absent or amount too small to determine enantiomeric composition)
[b] percentage approximate due to coelution with another compound

1997) and was detected in the captured volatiles of emerged and paired beetles of both sexes. It was 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 & Wood 1980; Byers 1983). The presence of verbenone in the volatiles of female *D. ponderosae* that were alone in a log (Figs 1, 3; Table 4) suggests that it may also 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 & Wood 1980; Byers 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 autoxidation of α-pinene as well as metabolic conversion by beetles and particularly microorganisms (Hunt *et al.* 1989; Hunt & 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-Phenylethanol has been reported in *Ips* and *Dendroctonus* spp. and increased the attraction of *I. paraconfusus* to logs infested by males (Renwick *et al.* 1976). 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 (Fig. 6).

Table 4 Mean amount ($\pm$SE) of volatiles in *D. ponderosae* excised from the field attacked tree 1–5 days after attack. For females, means followed by asterisks are significantly different, Wilcoxon's rank sum test, $P<0.05$

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

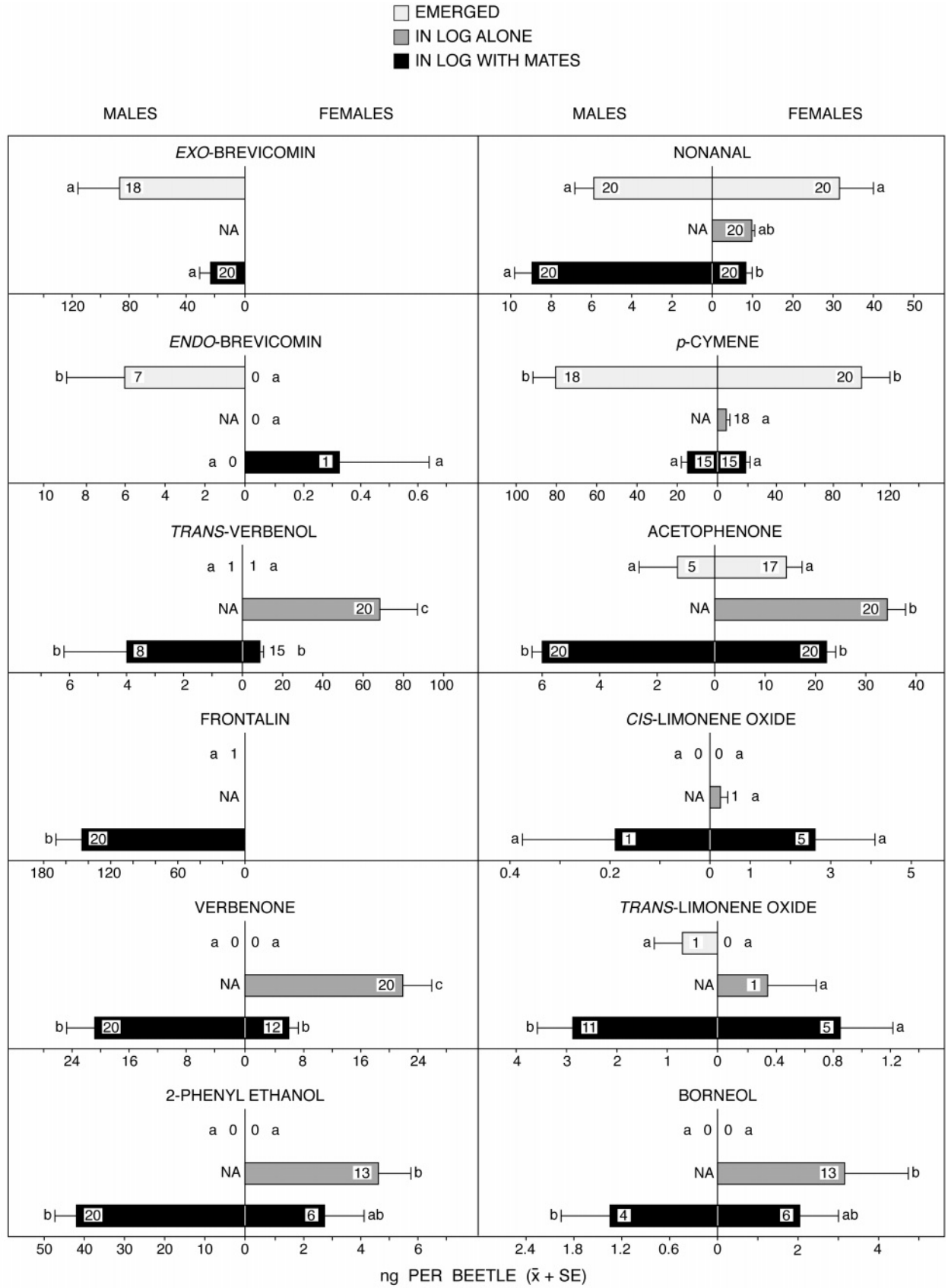

Fig. 3 Mean amounts of antennally-active compounds as determined by gas chromatographic analyses of individual extracts from 20 male and 20 female *D. ponderosae* at each of two or three attack phases, respectively. Note variable scales for amounts per beetle. Bars with the same letter are not significantly different, nonparametric multiple comparisons, $P < 0.05$. Numbers in or beside bars indicate number of beetles containing detectable amounts of compound. NA = Not Applicable

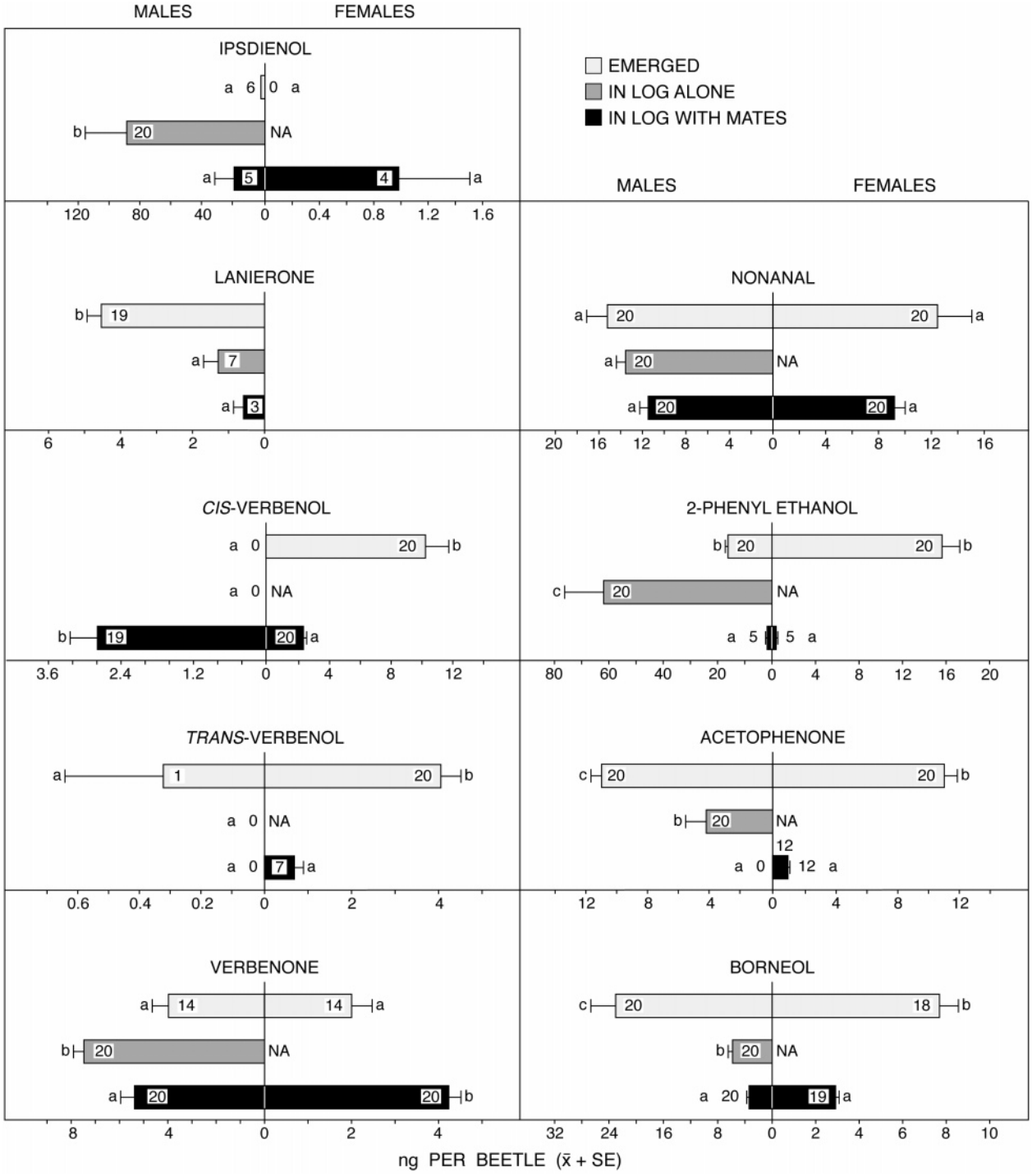

Fig. 4 Mean amounts of antennally-active compounds as determined by gas chromatographic analyses of individual extracts from 20 male and 20 female *I. pini* at each of three or two attack phases, respectively. Note variable scales for amounts per beetle. Bars with the same letter are not significantly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

It is a metabolite of phenylalanine in bark beetles and their yeasts (Leufvén *et al.* 1984). As a fermentation product, it indicates advanced microbial activity (Ishikawa *et al.* 1983). Our results suggest that it functions as an antiaggregation pheromone in *D. ponderosae*.

Nonanal, produced by both sexes of *D. ponderosae* at all phases of attack deterred the capture of *I. pini* in pheromone-baited traps (Fig. 6), 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). Our finding that it is a normal constituent of

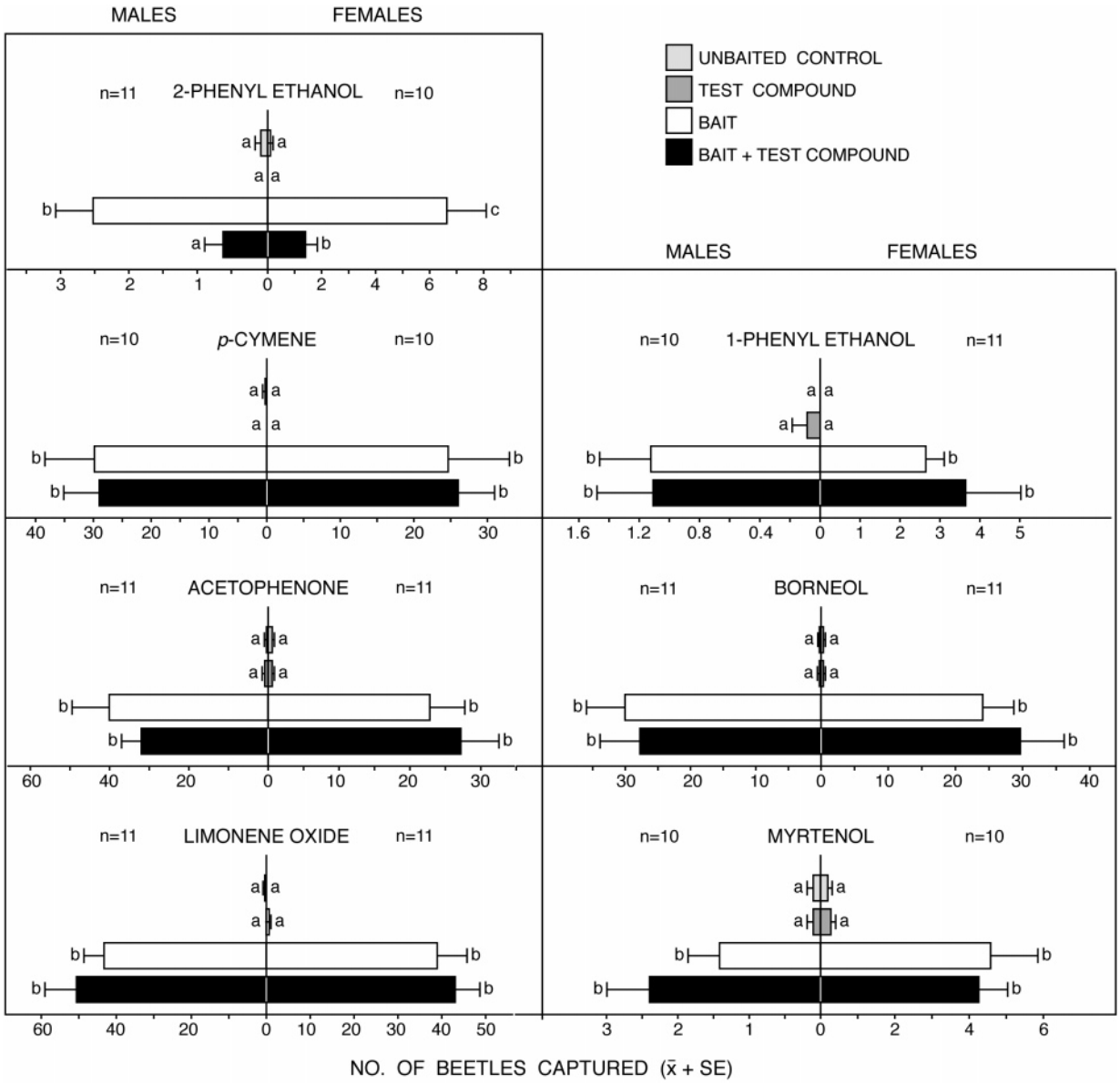

Fig. 5 Effect of previously untested antennally active compounds on the numbers of *D. ponderosae* captured in multiple funnel traps near Princeton B.C. between May and August 1998. Note variable scale for numbers of beetles captured. Bars (n = number of replicates) with the same letter are not significantly different, REGW multiple range test, $P < 0.05$

lodgepole pine volatiles suggests that it 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 & 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*-brevicomin, frontalin, 2-phenylethanol, verbenone and possibly nonanal, that emanate from the galleries as attack progresses.

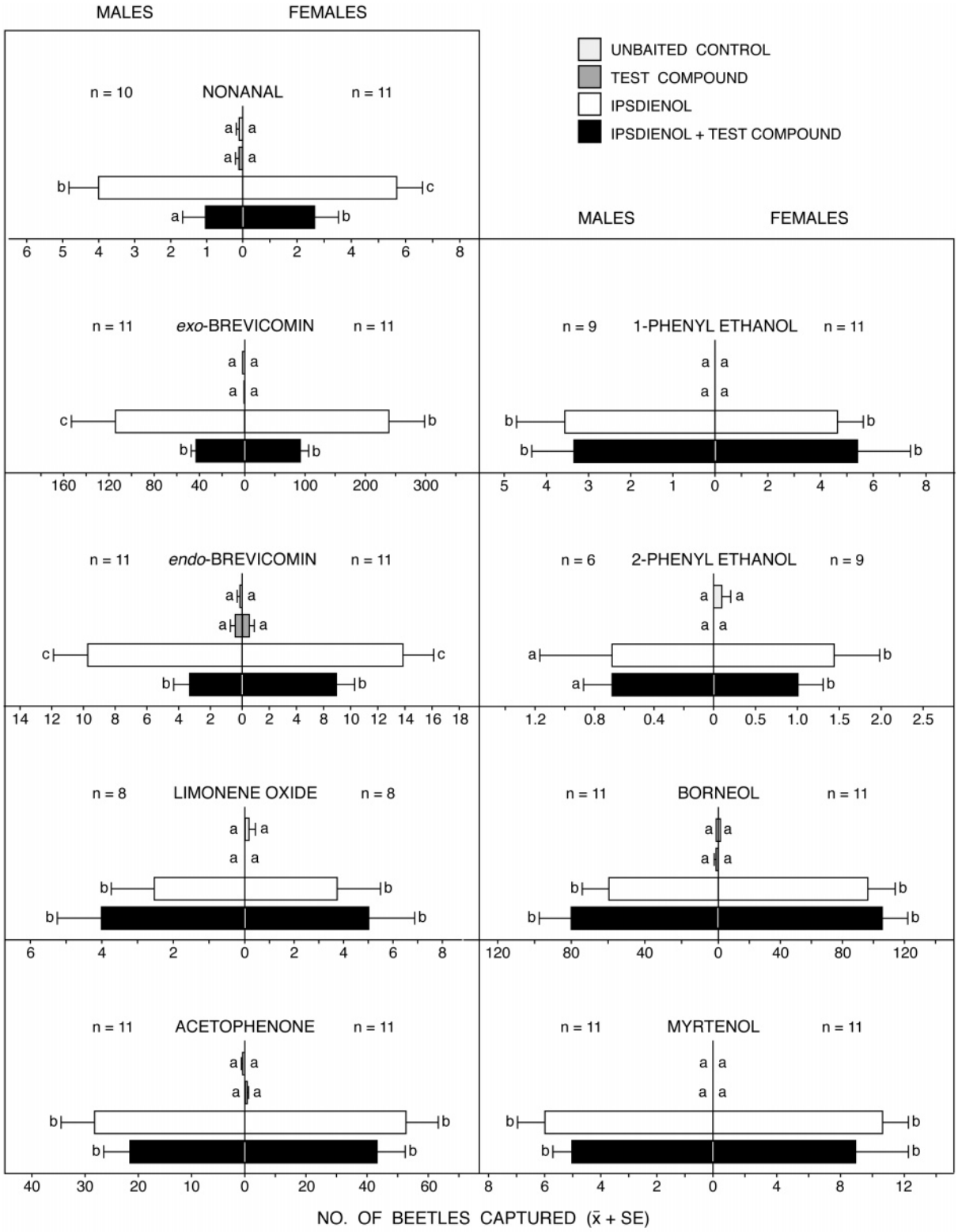

Fig. 6 Effect of previously untested antennally active compounds on the numbers of *I. pini* captured in multiple funnel traps near Princeton B.C. between May and August 1998. Note variable scale for numbers of beetles captured. Bars (n = number of replicates) with the same letter are not significantly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

Volatile production by 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 (Figs 2, 4) are consistent with observations on quantitative variation in aggregation pheromone production in male *I. typographus* (Bir-

gersson *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 & Vité 1972; Hughes 1973a,b; Elliott *et al*. 1975; Gore *et al*. 1977; Byers 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 & Borden 1994), were captured in the volatiles of emerged beetles and detected in the extracts of both sexes at all phases (Figs 2, 4), suggesting a role in inducing dispersal in emerged beetles. *cis*-Verbenol and verbenone may serve as antiaggregation pheromones that regulate attack density in *I. paraconfusus* (Byers & 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-Phenylethanol was repellent to *D. ponderosae* in the field (Fig. 5). As it was present in the volatiles of beetles at all attack phases and in the extracts of male *I. pini* that were in the log alone, it is probably used as a synomone that mediates resource partitioning between the two species. Gries *et al*. (1990a) detected 2-phenylethanol in extracts of wild and axenically-reared *I. pini*, but its biological activity was long unknown (Ivarsson & Birgersson 1995).

Acetophenone was present in all 40 emerged *I. pini* (Fig. 6). 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 & 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 behaviorally active.

Nonanal significantly inhibited the response of *I. pini* to ipsdienol (Fig. 6). Whether nonanal is sequestered or synthesised by *I. pini*, is as yet unknown. *endo*-Brevicomin, which is not an aggregation pheromone of *D. ponderosae* (Rudinsky *et al*. 1974c), inhibited the response of *I. pini* to ipsdienol (Fig. 6), contributing to the results of Miller (1990), wherein ipsenol, *exo*-brevicomin or a mixture of *cis*- and *trans*-verbenol disrupted the response of *I. pini* to ipsdienol. The strongly repellent nature of *exo*-brevicomin released at 3.1 mg per 24 h, to male *I. pini* confirms Miller's (1990) finding of a weakly dose-dependent inhibition of attraction to this compound. The ability of *I. pini* to detect only the (+) enantiomers of both *exo*- and *endo*-brevicomin is consistent with the predominance of these enantiomers in nature (Table 3) (Schurig *et al*. 1983). These results confirm that *exo*- and *endo*-brevicomin are synomones that would benefit both species in resource partitioning.

Attack dynamics

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 the production of common volatile compounds (Table 2) serving as semiochemicals (Hughes 1973a; White *et al*. 1980). 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 & Wood 1980; Byers 1983) and ensure outbreeding. Once a suitable host is located, aggregation pheromones produced by both species would simultaneously recruit conspecifics to the tree (Pitman *et al*. 1969; Billings *et al*. 1976; McKnight 1979; Borden *et al*. 1983; Conn *et al*. 1983; Libbey *et al*. 1985) and 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*.

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Tech Inc., Riverside Forest Products Ltd., Slocan Forest Products Ltd., TimberWest Ltd., Tolko Industries Ltd., Weldwood of Canada Ltd., West Fraser Mills Ltd., Western Forest Products Ltd. and Weyerhaeuser Canada Ltd.

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