THE ROLE OF KAIROMONES AND PHEROMONES IN HOST SELECTION BY TREE-KILLING BARK BEETLES (COLEOPTERA: SCOLYTIDAE)

by

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The role of kairomones and pheromones in host selection by tree-killing bark beetles (Coleoptera: Scolytidae)

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species decreased the response of Douglas-fir beetles, mountain pine beetles, and spruce beetles to their aggregation pheromones. Acetophenone, in volatiles of females of all species decreased the response of female Douglas-fir beetles, *trans*-Verbenol, an aggregation pheromone of the mountain pine beetle, decreased the response of Douglas-fir beetles, and 3-methyl-2-cyclohexen-1-one (MCH), the antiaggregation pheromone of the Douglas-fir beetle and spruce beetle, decreased the response of mountain pine beetles to aggregation pheromones. My results indicate that some (but not all) tree-killing bark beetles utilise host volatiles to discriminate between host and nonhost conifers, but suggest that most species can perceive volatiles from heterospecific beetles that attack nonhosts, and potentially use them to avoid attacking nonhost conifers.

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1. General introduction and thesis objectives

Bark beetles (Coleoptera: Scolytidae) feed and breed in the phloem of conifers throughout North America (S.L. Wood, 1982). They are generally specific to the tree species they commonly attack, as they are adapted to overcome the defenses of specific hosts, and to survive and develop within them. Pioneer beetles locate suitable hosts and the rest of the population follows in response to aggregation pheromones produced by pioneers (D.L. Wood, 1982; Borden *et al.*, 1986; Raffa *et al.*, 1993). Dispersing pioneers must therefore discriminate among sympatric species of conifers. Two hypotheses have been proposed regarding host location by pioneer beetles. The random landing hypothesis states that beetles land randomly on trees and select hosts at close range, after sampling them for suitability (Vité & Gara, 1962; Elkinton & Wood, 1980; Hynum & Berryman, 1980; Moeck *et al.*, 1981). Beetles may then rely on short-range olfactory cues or gustatory cues to accept or reject hosts (Doskotch & Chatterji, 1970; McNee *et al.*, 2003). The primary attraction hypothesis states that beetles locate hosts by long range perception of and response to volatile chemicals emanating from trees (McMullen & Atkins, 1962; Chapman, 1963; Austara *et al.*, 1986; Gries *et al.*, 1989; Moeck & Simmons, 1991; Byers, 1995; Brattli *et al.*, 1998).

In British Columbia (B.C.), four major species of tree-killing bark beetles are generally specific to the host species they attack (S.L. Wood, 1982). The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, attacks Douglas-fir, *Pseudotsuga menziesii*

(Mirb.) Franco. The mountain pine beetle, *D. ponderosae* Hopkins, attacks all species of pines, but most frequently attacks and kills lodgepole pine, *Pinus contorta* var. *latifolia* Engelm. The spruce beetle, *D. rufipennis* Kirby, attacks Engelmann spruce, *Picea engelmanii* Parry ex Engelm., white spruce, *P. glauca* (Moench) Voss, and their hybrid "interior" spruce, *P. engelmannii* x *glauca* (Wright, 1955), which occurs throughout most of B.C. The western balsam bark beetle, *Dryocoetes confusus* Swaine (hereafter abbreviated as *Dr. confusus*), attacks subalpine fir, *Abies lasiocarpa* (Hooker) Nutall, Rocky Mountain alpine fir, *A. bifolia* A. Murray, and their hybrid "interior" fir, *A. lasiocarpa* x *bifolia.* Distinct morphological and biochemical differences between *A. lasiocarpa* and *A. bifolia* justify separation of the species even though neither *A. bifolia* nor the hybrid interior fir, are uniformly accepted (Hunt, 1993). The two species of *Picea* and *Abies,* and their respective hybrids, are all wide-spread in the interior of B.C., and form the principal hosts of their respective bark beetles. For convenience, the "interior" hybrids are hereafter referred to as species.

Chemical cues that bark beetles encounter during host selection are complex (Borden *et al.*, 1986). They include: 1) attractive volatiles from hosts, 2) volatiles from hosts accentuated by volatiles emitted by conspecifics attacking hosts, 3) repellent volatiles from nonhosts, and 4) volatiles from nonhosts accentuated by volatiles emitted by sympatric heterospecific bark beetles attacking nonhosts. In this thesis, I use the terms pheromone and kairomone to refer to message-bearing chemicals with intra- and interspecific functions, respectively, the latter being beneficial to the receiver of the signal (Norlund, 1981).

Host monoterpenes are attractive to scolytids (Rudinsky, 1966a). Myrcene is a kairomone for *D. ponderosae* (Billings *et al.*, 1976; Conn *et al.*, 1983; Libbey *et al.*, 1985; Borden *et al.*, 1987a), and synergises the aggregation pheromone signal in traps. β-Phellandrene increased the attraction of the pine engraver, *Ips pini* (Say), to its aggregation pheromone ipsdienol (Miller & Borden, 1990). Compounds distilled from the phloem oil of *A. lasiocarpa,* were attractive to *Dr. confusus* (Camacho *et al.*, 1998), suggesting that beetles use them in host location. Response to aggregation pheromones emitted by conspecifics (secondary attraction) on initiation of attack is stronger than primary attraction to host volatiles alone (Person, 1931; Anderson, 1948; Rudinsky, 1966a,b; Borden, 1974; D.L. Wood, 1982).

Beetles may also be attracted to or repelled by pheromones of sympatric species of beetles attacking host trees (Svihra *et al.*, 1980; Poland & Borden, 1998a,b). When attracted to semiochemicals emitted by heterospecifics, beetles can use them to locate hosts which may be rare or patchy in distribution (Poland & Borden, 1994; Savoie *et al.*, 1998; Ayres *et al.*, 2001). Several studies also document perception and deterrence of bark beetles to semiochemicals emitted by heterospecifics inhabiting the same host species (Svihra *et al.*, 1980; Light *et al.*, 1983; Rankin & Borden, 1991; Borden *et al.*, 1992; Poland & Borden, 1998a,b; Savoie *et al.*, 1998; Pureswaran *et al.*, 2000; Ayres *et al.*, 2001). Behavioural response of bark beetles to volatiles emitted by heterospecific beetles attacking nonhosts, a signal that could potentially be used in nonhost avoidance during host selection, has not been investigated.

The chemical constitution of conifers has been elucidated, and most studies have focussed on either the monoterpene composition of foliage monoterpenes (von Rudloff, 1972a, 1975; Forrest, 1980; Zou & Cates, 1995) or xylem oleoresin (Mirov, 1961; Smith, 1983, 2000). Bark beetles can potentially orient toward or away from volatiles released from bark, wood and foliage of conifers. Bole volatiles include those from phloem and sapwood, both of which have interconnected tube-like resin ducts in pines, and cortical blisters in firs, that contain high amounts of monoterpenes (Chang, 1954; Fahn, 1979; Lewinsohn *et al.*, 1991). Several studies to date have shown that coniferophagous bark beetles can perceive and avoid volatiles from angiosperms in flight (Schroeder, 1988; Borden *et al.*, 1998; Zhang *et al.*, 1999; Huber & Borden, 2001a,b). However, the precise mechanisms by which beetles discriminate among sympatric species of conifers are not known.

The term host selection in my thesis refers to the process by which tree-killing bark beetles home in on the right species of conifer. The mechanisms by which bark beetles distinguish between suitable and unsuitable trees of the host species, is beyond its scope. My general objective was to investigate the mechanisms by which four major species of tree-killing bark beetles in B.C. discriminate among four sympatric species of conifers during host selection. In particular, my objectives were to:

1. determine whether volatiles from nonhost conifers were powerful enough to repel bark beetles from attacking nonhosts baited with their aggregation pheromones;

2. elucidate by gas chromatographic-electroantennographic detection analyses (GC-EAD), the suites of volatiles from bole and foliage of host and nonhost conifers, as well as con- and heterospecific beetles, that the four species of beetles can perceive and potentially use in host selection;

3. establish the extent to which the four species of conifers differed in their monoterpene profiles, and if there were intraspecifc differences among geographic locations in B.C.; and

4. ascertain by field experiments[1] whether the four species of beetles demonstrated
   - ◊ primary attraction to bole and foliage volatiles from hosts,
   - ◊ discrimination among bole and foliage volatiles from hosts and nonhosts, and / or
   - ◊ repellence to volatiles from heterospecific beetles that attack nonhosts.

[1] Efforts to trap *Dr. confusus* (objective 4) consistently yielded too few beetles for reliable statistical analyses. Therefore, Chapters 5 and 6 report results only for the three *Dendroctonus* spp. and results for *Dr. confusus* are summarised in the Appendix.

2. Test of semiochemical-mediated host specificity[2]

2.1. Introduction

Location of suitable hosts is vital to the reproductive success of tree-killing bark beetles (Raffa & Berryman, 1982a; D.L. Wood, 1982). Beetles may select hosts by random landing and testing at close-range (Vité & Gara, 1962; Elkinton & Wood, 1980; Hynum & Berryman, 1980; Moeck *et al.* 1981), or by long-range primary attraction to host volatiles (McMullen & Atkins, 1962; Chapman, 1963; Austara *et al.*, 1986; Gries *et al.*, 1989; Moeck & Simmons, 1991; Byers, 1995; Brattli *et al.*, 1998). An alternative hypothesis that would partially explain specificity in host selection is that beetles avoid nonhosts at long range, by perceiving volatiles that may be repellent. To test this hypothesis, I used host and nonhost trees baited with the respective aggregation pheromones of beetles in this study, to draw beetles into the area and force them to distinguish between pheromone-baited hosts and nonhosts. Pheromone-baited trees were used instead of unbaited trees, because of practical difficulties involving tracking and observation of beetles in the absence of pheromone baits.

My objectives were to determine whether under the influence of aggregation pheromones, 1) if any of the four species of bark beetles avoid nonhost conifers in flight

[2] This chapter has been published: Pureswaran D.S. and J.H. Borden (2003). Test of semiochemical mediated host specificity in four species of tree killing bark beetles (Coleoptera: Scolytidae). Environmental Entomology. 32: 963-969. Reproduced with permission from publisher.

as opposed to after landing, and 2) if volatiles from nonhost conifers can overpower an aggregation pheromone signal and prevent mass attack.

2.2. Materials and methods

Experimental design

Four 10 replicate experiments (Table 2.1.) conducted between April and August 1999 focussed on two ecological associations in which pairs of beetle species were sympatric and did not share aggregation pheromone components. *Dendroctonus ponderosae* and *D. pseudotsugae* were tested for discrimination between lodgepole pine and Douglas-fir, and *D. rufipennis* and *Dr. confusus* for discrimination between interior spruce and interior fir.

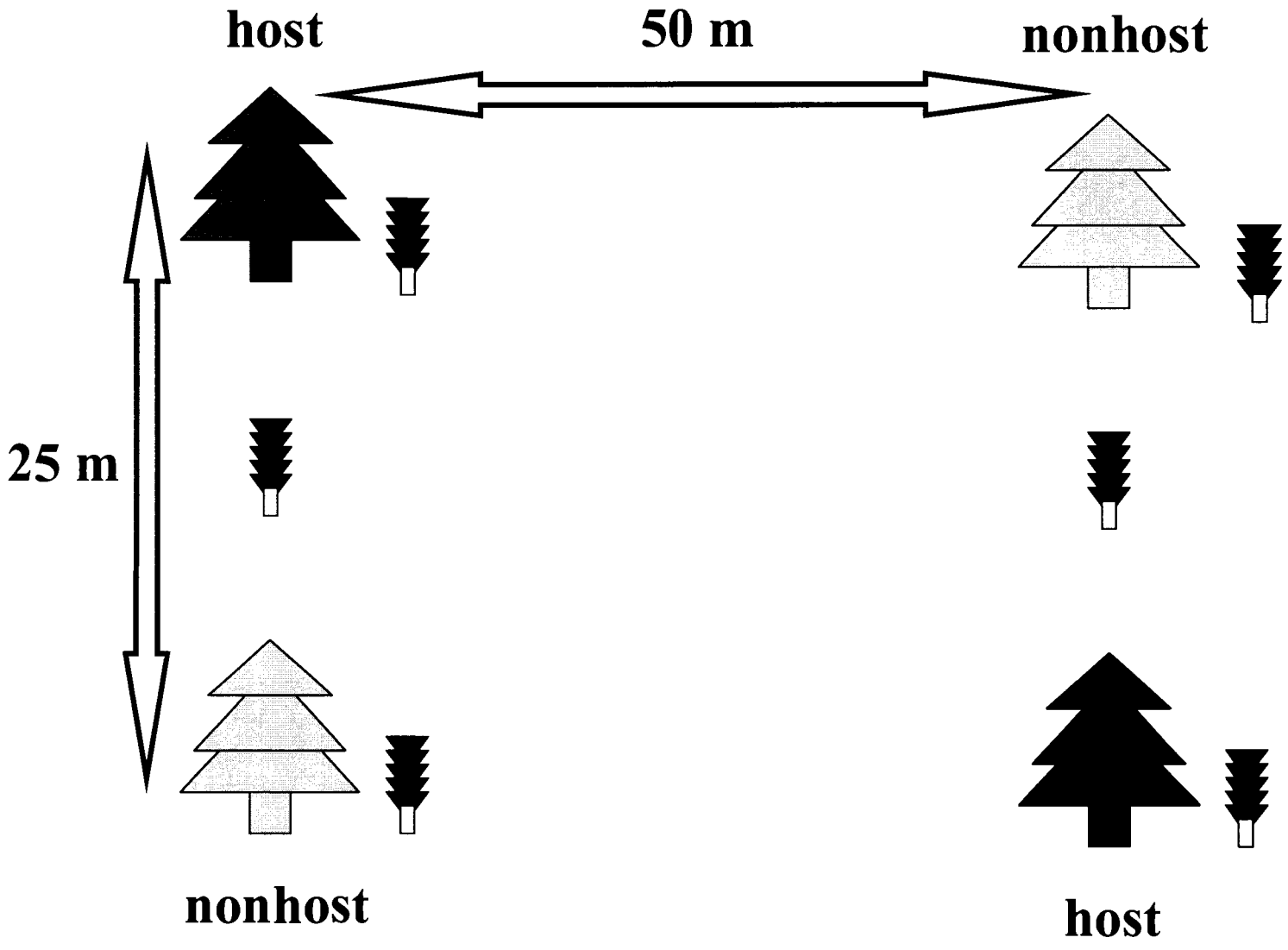
Experimental blocks (Figure 2.1.) were set up in interior B.C. locations where populations of both tree and beetle species were prevalent (Table 2.1.). The first tree species in each pair was randomly determined by flipping a coin. A suitable large tree ($\geq$ 25 cm diameter at 1.3 m) was then selected and a similar tree of the opposite species was chosen ca. 25 m away. If two such trees could not be found, a new search in a site nearby was initiated. The distance between replicates was $\geq$ 50 m. Aggregation pheromone baits were affixed to the north face of each tree. Unbaited multiple-funnel traps (Lindgren, 1983) (Phero Tech Inc., Delta, B.C.) were placed on the east face, 1 m from baited trees, to catch incoming flying beetles that oriented towards the tree. A piece of Vapona No-Pest Strip (Green Cross, Fisions Horticulture Inc., Mississauga, Ontario, Canada) was placed in the collecting cup to prevent escape of captured bark beetles, and to kill predatory beetles. Hardware cloth panels (20 x 50 cm) coated with Tangle trap®

Table 2.1. Description of locations and treatments in four paired-tree experiments in 1999 to test discrimination between host and nonhost conifers by four species of tree-killing bark beetles.

Exp. No.	Location in B.C. and duration	Tree species baited and diameter in cm (mean ± SE)	Species	Composition of pheromone bait[a]	Release device	Release rate (mg / 24h)[b]
1	Spring Lake Road, 30 km north of 100 Mile House, 7 May - 5 July	lodgepole pine (31.5 ± 1.6) and Douglas-fir (38.8 ± 1.7)	*Dendroctonus pseudotsugae*	(±)-frontalin	polyethylene microcentrifuge tube	2.6
2	Valentine Lake, 40 km west of 100 Mile House, 9 June – 14 September	lodgepole pine (27.3 ± 1.0) and Douglas-fir (40 ± 3.6)	*Dendroctonus ponderosae*	82% (-)-*trans*-verbenol, (±)-*exo*-brevicomin	bubble cap flex lure	1.5 0.28
3	Tulameen Forest Service Road, 50 km South of Merritt, 26 May – 18 August	Interior spruce (59.1 ± 4.3) and Interior fir (47.6 ± 5.3)	*Dendroctonus rufipennis*	(±)-frontalin	polyethylene microcentrifuge tube	2.6
4	Art Creek Road, 50 km east of 100 Mile House, 2 June – 31 August	Interior spruce (41.0 ± 2.5) and Interior fir (34.2 ± 1.9)	*Dryocoetes confusus*	(±)-*exo*-brevicomin	polypropylene microcentrifuge tube	1.7

[a] Pheromone baits were supplied and release rates determined by Phero Tech Inc., Delta, B.C. Commercial tree baits do not contain host monoterpenes.
[b]Release rate temperatures were: *trans*-verbenol at 20°C; *exo*-brevicomin, at 20°C; frontalin, at 23°C.

Figure 2.1. Diagrammatic representation of experimental design showing two replicates. Baited trees within a pair (replicate) were ca. 25 m apart, with $\geq$ 50 m between replicates. Unbaited multiple funnel traps were placed 1m away from each tree in a pair and equidistant between them.


host
50 m
nonhost
25 m
nonhost
host

(The Tanglefoot Company, Grand Rapids, MI 49504) were affixed to the west face of the baited tree to capture landing beetles. For each replicate, a third unbaited trap (a negative control) was placed mid-way between the baited trees.

At the end of the flight period, captured beetles from all traps and sticky panels were collected and the species, sex and number of all scolytids were determined (Lyon, 1958; Jantz & Johnsey, 1964; S.L. Wood, 1982). Both host and nonhost trees were visually assessed for attack from ground level to 2 m in height. Trees with ≥ 5 successful attacks on the north face of the tree, with copious frass and on pines and spruces abundant resin flow and pitch tubes were considered to be mass attacked. Five galleries (or fewer if < 5 attacks were present) were dissected on each tree and assessed for attack success by the presence or absence of boring adults, egg niches, eggs and larvae. The species of all adults recovered from the galleries was determined (S.L. Wood, 1982).

Statistical analyses

Catches from traps and sticky panels were transformed by $\log_{10}(x+1)$. Trap catch data were analysed by ANOVA (Proc GLM) (SAS Institute Inc., 1990), with treatment (traps associated with hosts, nonhosts and control traps) as the main effect, and the Ryan-Einot-Gabriel-Welsh Multiple Range (REGW) test (Day & Quinn, 1989), to determine whether there was a difference in the number of beetles captured in traps associated with host trees compared to nonhosts. Data from the sticky panels were analysed by t-tests (SAS Institute Inc., 1990) to determine if there was a significant difference in landing of beetles on hosts compared to nonhosts. In all cases $\alpha = 0.05$.

2.3. Results

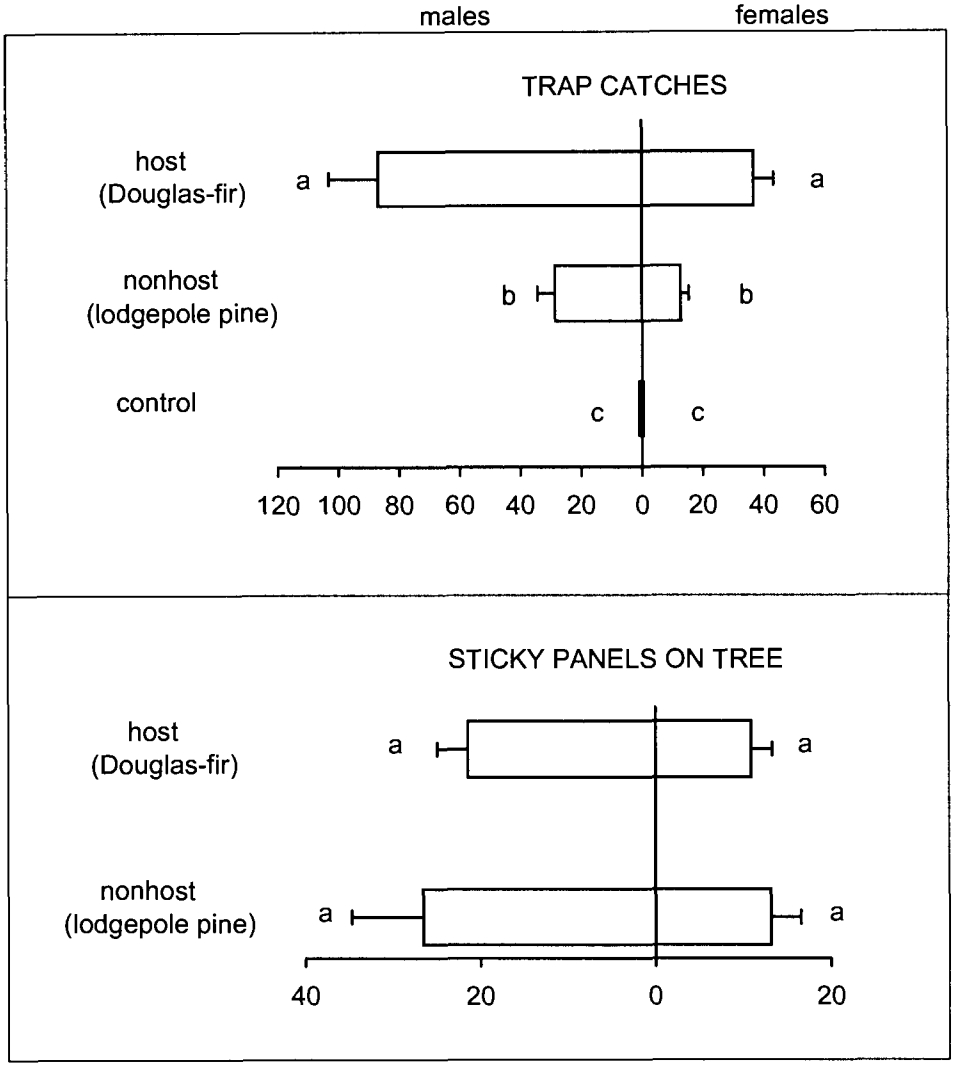
In almost all instances there was successful attack on host trees, but in no case was attack successful on nonhosts as assessed by brood in galleries (Table 2.2.). Neither *D. rufipennis* nor *Dr. confusus* initiated any attack on the lower 2 m of bark on either of their respective nonhosts. In contrast, on lodgepole pine, 50 attempted attacks by *D. pseudotsugae* were observed. Five of these reached the phloem tissue, but none contained eggs or larvae. Of the five galleries, four were on one tree and one on another. On Douglas-fir, none of the 39 observed attacks by *D. ponderosae* reached the phloem tissue. More *D. pseudotsugae* of both sexes were caught in traps beside nonhost lodgepole pines than in control traps, and more were caught beside Douglas-fir trees than lodgepole pines (males $F = 145.65$, $df = 2, 15$, $P < 0.0001$; females $F = 114.76$, $df = 2, 15$, $P < 0.0001$), but beetles landed equally on the sticky panels on both hosts and nonhosts (males $t = 0.22$, $df = 16$, $P = 0.83$; females $t = 0.06$, $df = 16$, $P = 0.95$) (Figure 2.2.). Although traps near the baited trees caught significantly more *D. ponderosae* than control traps, unlike *D. pseudotsugae*, there was no difference in their orientation towards traps near host lodgepole pines and nonhost Douglas-firs (Figure 2.3.). Landing of *D. ponderosae* on host and nonhost trees did not differ significantly (males $t = 0.04$, $df = 18$, $P = 0.97$; females $t = 0.63$, $df = 18$, $P = 0.54$).

There was no difference among catches of male *D. rufipennis* between control traps and traps near hosts and nonhosts (males $F = 1.94$, $df = 2, 13$, $P = 0.18$). The ANOVA detected a significant treatment effect in females (females $F = 4.43$, $df = 2, 13$,

Table 2.2. Comparison of attack success by *D. pseudotsugae*, *D. ponderosae*, *D. rufipennis* and *Dr. confusus* on hosts and nonhosts.

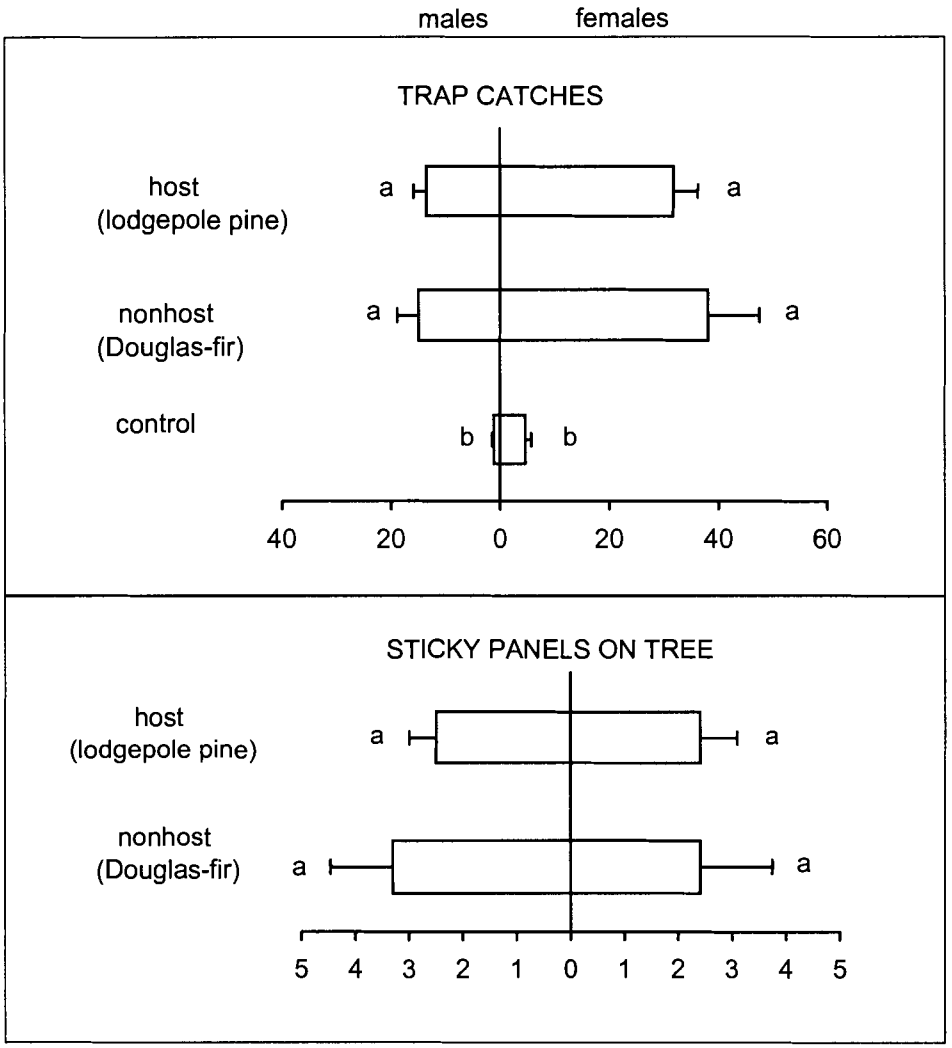
Criterion measured	*D. pseudotsugae*		*D. ponderosae*		*D. rufipennis*		*Dr. confusus*	
	host (Douglas-fir)	nonhost (lodgepole pine)	host (lodgepole pine)	nonhost (Douglas-fir)	host (interior spruce)	nonhost (interior fir)	host (interior fir)	nonhost (interior spruce)
No. trees	9	9	10	10	10	10	10	10
No. with attempted attack	9	8	10	9	9	0	10	0
No. mass attacked	9	0	10	0	8	0	8	0
No. galleries examined (hosts) or no. attempted attacks observed (nonhosts) upto 2 m height on bole	53	50	51	39	44	0	50	0
No. examined or observed galleries that penetrated to phloem	53	5	51	0	39	0	50	0
No. galleries with brood	48	0	51	0	37	0	30	0
Gallery length (cm) (mean $\pm$ SE)	13.2 $\pm$ 1.1	6.4 $\pm$ 1.4	23.1 $\pm$ 1.4	0	7.7 $\pm$ 0.6	0	3.4 $\pm$ 0.4	0

Figure 2.2. Numbers of *D. pseudotsugae* captured in unbaited control traps, pheromone baited traps associated with host and nonhost trees, and on sticky panels attached to host and nonhost trees. Bars within a subgraph with the same letter are not significantly different, REGW multiple range test (for trap catch data) and t-test (for sticky panel data) respectively. In all cases, $\alpha = 0.05$.



number of beetles captured (mean + SE)

Figure 2.3. Numbers of *D. ponderosae* captured in unbaited control traps, pheromone baited traps associated with host and nonhost trees, and on sticky panels attached to host and nonhost trees. Bars within a subgraph with the same letter are not significantly different, REGW multiple range test (for trap catch data) and t-test (for sticky panel data) respectively. In all cases, $\alpha = 0.05$.



number of beetles captured (mean + SE)

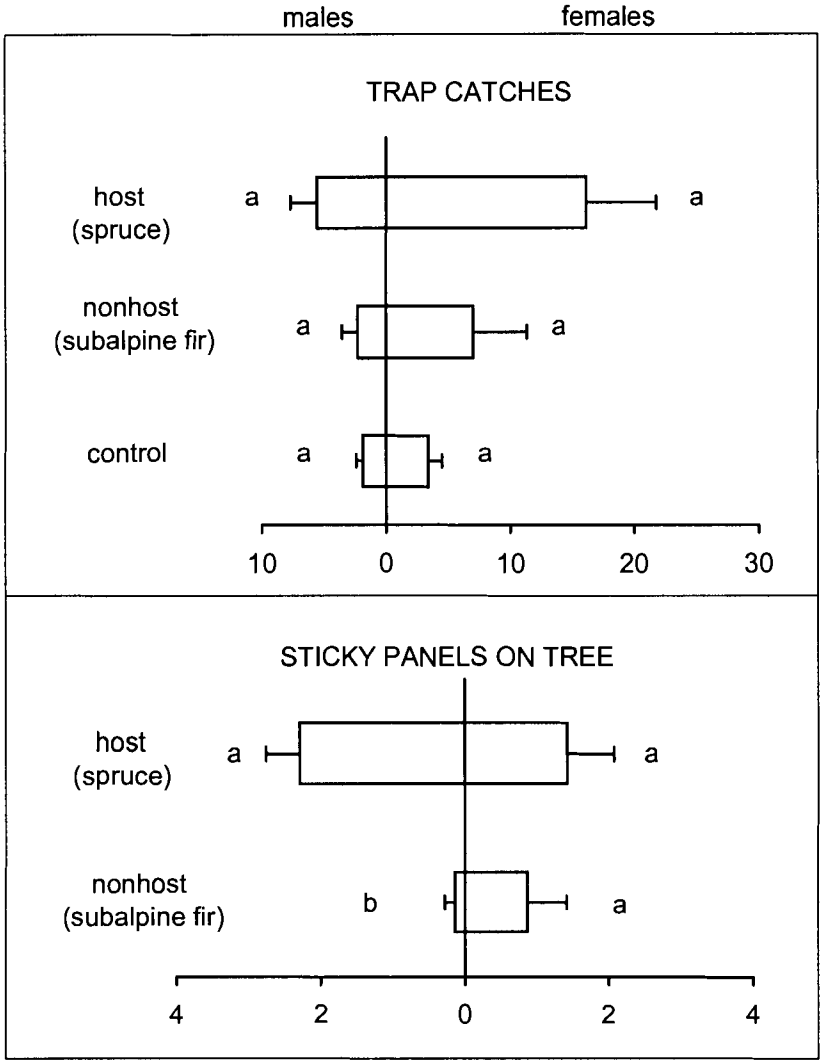
$P = 0.03$), but the more conservative REGW multiple range test did not detect a difference among treatments (Figure 2.4.). Male *D. rufipennis* landed preferentially on hosts, while females failed to distinguish between hosts and nonhosts (males $t = 5.95$, $df = 12$, $P < 0.0001$; females $t = 0.85$, $df = 12$, $P = 0.41$). Male *Dr. confusus* were caught in greater numbers in traps near nonhosts than in control traps, and both sexes were caught (males $F = 9.82$, $df = 2, 16$, $P = 0.0016$; females $F = 13.12$, $df = 2, 16$, $P = 0.0004$) and landed (males $t = 3.42$, $df = 18$, $P = 0.0031$; females $t = 4.15$, $df = 18$, $P = 0.0006$) in greater numbers on hosts than on nonhosts (Figure 2.5.).

2.4. Discussion

Lack of any attacks by *D. rufipennis* and *Dr. confusus* on nonhosts, and very small numbers of attacks by *D. pseudotsugae* and *D. ponderosae* that even reached the phloem tissue indicates a very low likelihood that orientation toward or landing on nonhost trees was influenced by supplementary aggregation pheromones produced by attacking beetles. Therefore any positive response (orientation or landing) on nonhosts by any of the four species can be attributed to a response to the aggregation pheromone baits.

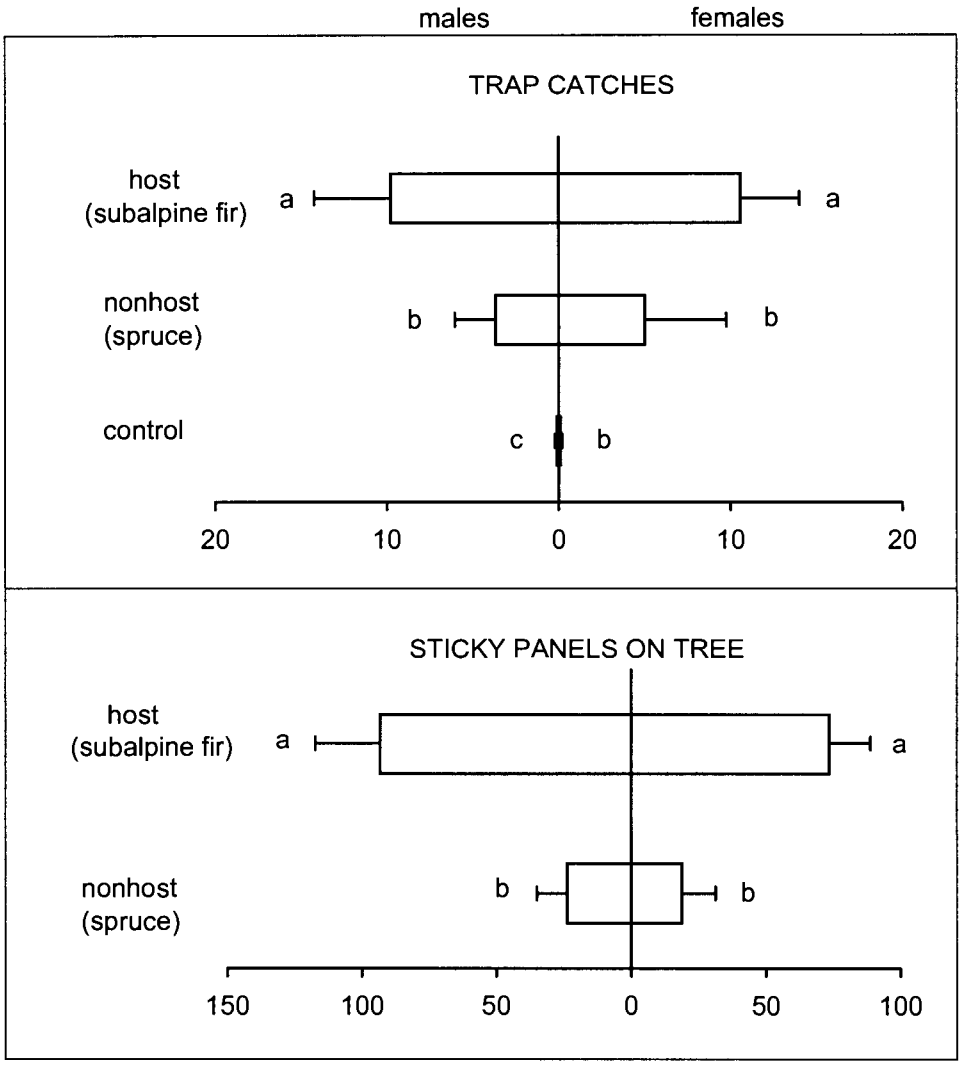
Significant trap catches of *D. pseudotsugae*, *D. ponderosae,* and (first attacking) male *Dr. confusus* on traps adjacent to nonhost trees suggest that none of these species was strongly repelled by its sympatric nonhost conifer, at least to the extent that nonhost volatiles released by an undamaged tree could overcome an attractive pheromone bait. This conclusion is reinforced by the lack of significant differences in landing rates under the influence of pheromone, on hosts and nonhosts by *D. pseudotsugae*, *D. ponderosae*,

Figure 2.4. Numbers of *D. rufipennis* captured in unbaited control traps, pheromone baited traps associated with host and nonhost trees, and on sticky panels attached to host and nonhost trees. Bars within a subgraph with the same letter are not significantly different, REGW multiple range test (for trap catch data) and t-test (for sticky panel data) respectively. In all cases, $\alpha = 0.05$.



number of beetles captured (mean + SE)

Figure 2.5. Numbers of *Dr. confusus* captured in unbaited control traps, pheromone baited traps, and on sticky panels attached to host and nonhost trees. Bars within a subgraph with the same letter are not significantly different, REGW multiple range test (for trap catch data) and t-test (for sticky panel data) respectively. In all cases, $\alpha = 0.05$.



number of beetles captured (mean + SE)

and female *D. rufipennis*. These results lend support to the random landing hypothesis (Vité & Gara, 1962; Elkinton & Wood, 1980; Hynum & Berryman, 1980; Moeck *et al.*, 1981), although they do not consider the other argument for random landing, the absence of primary attraction.

Byers' (1996) model suggests that primary attraction to host volatiles is not imperative for host location in many species of bark beetles. *Dendroctonus ponderosae* is the most aggressive of the beetles in this study, and in the absence of aggregation pheromone baits, it landed at similar rates on hosts and nonhosts (Hynum & Berryman, 1980; Moeck *et al.*, 1981). However, Moeck & Simmons (1991) demonstrated that cages baited with fresh lodgepole pine bolts or wood attracted more beetles than empty control cages, suggesting moderate primary attraction to host odours. Similar results were obtained by Chapman (1963) for *D. pseudotsugae,* and Stock & Borden (1983) for *Dr. confusus*. However, in all three studies, the host bolts were freshly cut and unsealed, and may have emitted a stronger olfactory signal than an intact, standing tree.

Hart's (1987) cladogram of the Pinaceae shows that pines and spruces, the hosts of two of the three *Dendroctonus* spp. in my study, are more closely related to each other, than they are to the *Abies* host of *Dr. confusus*. Although *Dr.confusus* could have been repelled by nonhost spruce trees, its *Abies* host contains over five times more bole volatiles than any of the other three species of conifers in this study (Chapter 4). Hence, due to an increased threshold of perception for host volatiles, *Dr. confusus* simply may not perceive volatiles from other conifers.

Under the influence of aggregation pheromone baits, equal landing rates on hosts and nonhosts by *D. ponderosae* and *D. pseudotsugae*, as well as female *D. rufipennis* in this study could have occurred because beetles that successfully attacked their host trees produced antiaggregation pheromones that neutralized the attractive baits. The high landing rates by male *D. rufipennis* on hosts may have been a response at close range to the presence of females. Stock *et al.* (1990), concluded that (+)-*endo*-brevicomin alone was an antiaggregation pheromone for *Dr. confusus*. It was later shown to be an aggregation pheromone component in combination with (+)-*exo*-brevicomin (Camacho *et al.*, 1993). Lack of an antiaggregation pheromone would explain the superior trap catches and landing rates by this beetle on its host tree.

As demonstrated in *Scolytus multistriatus* (Marsham) and *Scolytus quadrispinous* Say (Norris & Baker, 1967; Baker & Norris, 1968a,b; Gilbert & Norris, 1968), *D. pseudotsugae* and *D. ponderosae* may detect nonhosts at close range, possibly after attempting to initiate attack, as was evident by the failed attacks on baited nonhosts. However, the lack of abundant and / or partially successful attacks by any of the four species on nonhosts dispels the frequently asked question of whether baiting nonhost trees with aggregation pheromones is a potential management tactic, similar to Smith's (1986) proposal of using baited hosts treated with insecticide. Although nonhost trees that were baited with pheromones did not entirely repel *Dendroctonus* spp., attack was never successful, and most beetles that landed on the wrong host would probably have left on finding it unsuitable. The possibility of managing them by inducing landing and some attack on nonhosts treated with topical, but not systemic insecticide (no significant

feeding was observed on nonhosts) remains. However, successful management would be most likely with host trees used in trap- or lethal-tree tactics.

In conclusion, this study does not reveal long range repellent effects of any nonhost tested. Significant trap catches, landing rates and a few attempts at gallery initiation into nonhosts by *D. pseudotsugae* and *D. ponderosae* indicate that nonhost volatiles did not overpower the effect of aggregation pheromones. A similar test by Byers *et al.* (2000) demonstrated that *Pityogenes bidentatus* (Herbst) that colonizes the limbs of weak Scots pine, *Pinus sylvestris* L., attempted unsuccessful attacks on aggregation pheromone-baited nonhost birch, *Betula pendula* Roth. This suggests that as reported in the California fivespined ips, *Ips paraconfusus* Lanier (Elkinton & Wood, 1980), nonhost rejection and host acceptance may be based on gustatory stimuli. No attack on nonhosts was observed by *D. rufipennis* and *Dr. confusus*, even though they landed on sticky panels in response to the attractive pheromone baits. The attractive power of the pheromone baits in the experiment would have forced incoming beetles that were not captured, to detect and avoid nonhosts at close range. In the absence of pheromones, these two species may be able to avoid nonhosts at long range in nature.

3. Antennal responses of bark beetles to volatiles from host and nonhost conifers, and con- and heterospecific beetles

3.1. Introduction

In Chapter 2, I found that nonhost volatiles did not inhibit orientation of beetles toward pheromone-baited nonhosts. *D. pseudotsugae* and *D. ponderosae* oriented toward, landed on, and initiated attack on pheromone-baited nonhosts. *D. rufipennis* and *Dr. confusus* oriented and landed on pheromone-baited nonhosts, but did not initiate attack on them. I hypothesised that this may be due to 1) differences or similarities in the volatile profiles of the four species of conifers, and 2) differences in the ability of beetles to perceive differences in volatiles in the four sympatric species of conifers.

Using gas chromatographic-electroantennographic detection analyses (GC-EAD)[3], I compared the electrophysiological detection capacity of males and females of the four species of bark beetles to 1) volatiles from the bole and foliage of the four species of conifers and 2) volatiles emitted by males and females of each of the four species of bark beetles at three phases of attack, against the antennae of males and females of the four species. I collected volatiles from beetles at different phases of attack to ensure that I did not miss any compound that was absent or present in minute amounts at any stage of attack. GC-EAD technology helps to identify particular chemicals that stimulate the antennae of beetles, out of a whole array of chemicals that are present in

volatiles of trees and beetles. My goal was to determine 1) the range of volatiles that beetles could perceive from conifers, as well as from con- and heterospecific beetles, and 2) whether there were species-specific responses by beetles, to compounds in conifers. Volatiles identified by GC-EAD, are candidate semiochemicals with potential behavioural activity (tested in the field in Chapters 5 and 6), and can be used in host location.

3.2. Materials and Methods

Collection of volatiles from conifer bole and foliage

Two trees (> 25 cm diameter at 1.3 m) of each species were felled in different locations in British Columbia (B.C.). Douglas-fir was felled on 28 May, 1999 at the Malcolm Knapp Research Forest, Maple Ridge. Lodgepole pine was felled on 30 April 1998 at Sunday Summit, 30 km south of Princeton. Interior spruce was felled on 10 May, 1999 on Spring Lake Road, 20 km north of 100 Mile House. Interior fir was felled on 17 July 1999 on Canim Lake Road, 40 km east of 100 Mile House. Branches bearing foliage from mid-crown were harvested; the bole was sawn into bolts 60 cm long, and transported to the laboratory. Within 48 h, the bolts were sawn into discs ~ 4 cm thick. Two discs from each species, and about 400 g of foliage, were placed in separate 10 L plastic chambers, and aerated for 24 h at 25°C with an airflow of ca. 2.5 L/min. Volatiles were captured in a glass column (14 mm OD, 20 cm long) packed with Porapak-Q (50-80 mesh, Waters Associates Inc., Milford, MA 01757) (Byrne *et al.*, 1975), and eluted with

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

150 mL of distilled pentane. Volatiles were concentrated to 4 mL by distillation of solvent through a 30 cm long Dufton column. Volatiles from both trees of each species, were combined for use in gas chromatographic-electroantennographic detection (GC-EAD) analyses.

Collection of volatiles from beetles

Trees naturally infested with beetles (downed trees for *D. pseudotsugae*), and accompanying uninfested trees of each species, were felled in interior B.C. locations as follows: *D. pseudotsugae,* 16 February, 1999, Cowichan Lake on Vancouver Island, and 2 February, 2000, Malcolm Knapp Research Forest at Maple Ridge; *D. ponderosae,* 6 May, 1998, Sunday Summit, 30 km south of Princeton; *D. rufipennis,* 10 May 1999, Spring Lake Road, 20 km north of 100 Mile House; and *Dr. confusus,* Art Creek Road, 5 November, 1999, 50 km east of 100 Mile House. Infested bark was removed from Douglas-firs. For other species, infested trees were bucked into bolts 60 cm long. Cut ends of all bolts were sealed with paraffin to minimise desiccation. Infested bolts or bark were placed in screened cages at 27°C and beetles collected on emergence. To obtain beetles from different stages of attack, they were treated as follows: 1) freshly emerged males and 2) females, 3) first attacking sex (females for *Dendroctonus* spp. and males for *Dr. confusus*) alone allowed to mine in an uninfested bolt for 24 h, 4) paired males (for the monogamous *Dendroctonus* spp.) or males with mates (for the polygamous *Dr. confusus*) and 5) paired females. For the treatments 4 and 5 with paired beetles, mates

were supplied after 24 h of mining. Beetles were excised after 48 h of attack for treatment 3 and after 96 h for treatments 4 and 5 (Pureswaran *et al.*, 2000).

Fifty *D. ponderosae* and 70 beetles of each sex of the other three species from each stage of attack were aerated as a group in Pyrex® tubes (1.2 cm OD, 18 cm long) until most of the beetles died. A modified version of the apparatus developed by Rudinsky *et al.* (1973) was used (Gries *et al.*, 1992), with air drawn at 1.5 L / min through a charcoal filter, the Pyrex tube, and finally a glass column (6 mm OD, 15 cm long) containing 3 cm of Porapak-Q (50-80 mesh) (Byrne *et al.*, 1975). Volatiles were flushed out with 1 mL of pentane using nitrogen gas.

Gas chromatographic-electroantennographic detection analyses (GC-EAD) and GC-mass spectrometry (GC-MS)

Bole and foliage volatiles from the four species of conifers, as well as volatiles from beetles of all four species at each stage of attack were analysed by GC-EAD (Arn *et al.*, 1975) against the antennae of freshly emerged males and females of each of the four species of beetles. Volatiles were run simultaneously past an antenna, and through the flame ionisation detector of a Hewlett Packard 5890 gas chromatograph fitted with a fused silica column (DB-5, 30 m x 0.32 ID, J&W Scientific, Folsom, CA 95630). Compounds that elicited an antennal response were analysed by coupled GC-mass spectrometry (MS) (Varian Saturn Ion Trap). The temperature programme was 50°C for 1 min and then 10°C / min to 280°C. Injector and detector ports were held at 250°C and 260°C, respectively. Helium was the carrier gas, with a flow rate of 1 cubic cm per min.

Spectral comparisons with authentic standards and calculated retention indices (van den Dool & Kratz, 1963) were used to identify compounds.

Enantiomeric ratios of chiral compounds in the conifers were analysed on a cyclodex B column (30 m x 0.25 mm ID, J&W Scientific, Folsom CA 95630-4714). The settings were 80°C for 12 min., increased at 5°C / min. to 150°C held for 5.5 min. Quantities of compounds found in volatiles of beetles were too low to enable analyses for enantiomeric composition. Candidate semiochemicals for future behavioural experiments were restricted to those that elicited peaks on antennograms that were equal to or greater in height than a fifth of the highest peak.

3.3. Results

Antennal responses to volatiles from conifers

Figure 3.1. presents a sample chromatogram and antennal responses of female and male beetles of all four species to the captured volatiles of interior spruce bole. Thirteen compounds that were antennally active were identified in the volatiles of all four species of conifers (Table 3.1.). Volatiles from all four species of conifers were qualitatively similar. All species of beetles detected seven of the 13 compounds. *Dendroctonus pseudotsugae* and *D. ponderosae* detected 12, while *D. rufipennis* and *Dr. confusus* detected 10 of the compounds. Eight of these compounds were chiral, with the (–)-enantiomer predominating over its antipode, except for 3-carene, which occurred primarily in the (+) form (Table 3.2.). More (+)-α-pinene occurred in the foliage of

Figure 3.1. Representative responses (EAD) of both sexes of four species of tree-killing bark beetles to spruce bole volatiles (FID). Identified FID peaks are (1) α-pinene, (2) sabinene, (3) β-pinene, (4) myrcene, (5/6) limonene and β-phellandrene, (7) nonanal

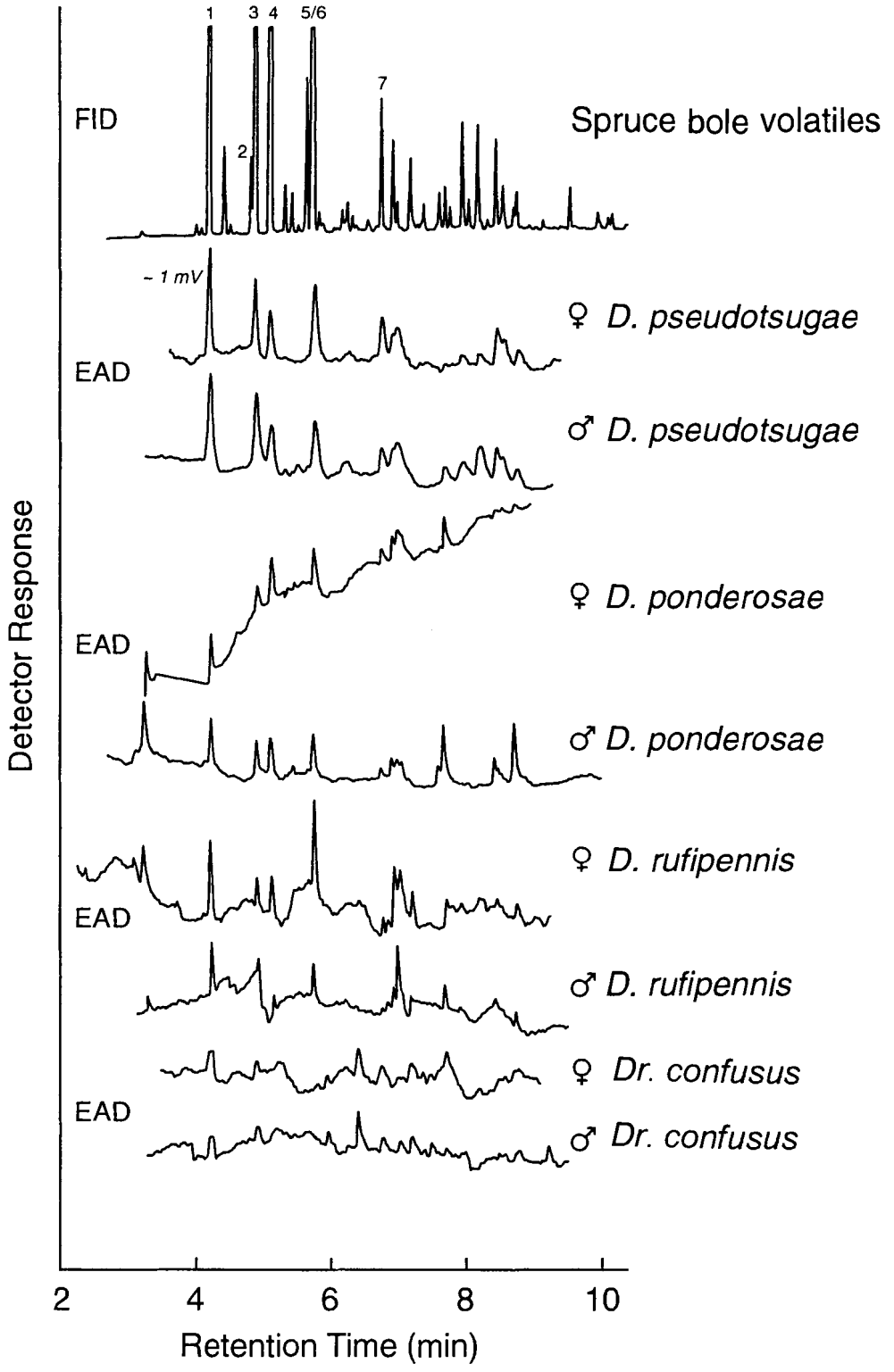

1
3 4
5/6
7
2
FID
Spruce bole volatiles
~ 1 mV
EAD
♀ D. pseudotsugae
♂ D. pseudotsugae
♀ D. ponderosae
EAD
♂ D. ponderosae
♀ D. rufipennis
EAD
♂ D. rufipennis
♀ Dr. confusus
EAD
♂ Dr. confusus
Detector Response
2
4
6
8
10
Retention Time (min)

Table 3.1. Antennal responses of four species of tree-killing beetles to monoterpenes in the volatiles from bole and foliage of four species of conifers.

	Antennal response (+) or no response (NR)			
Compound	*Dendroctonus pseudotsugae*	*Dendroctonus ponderosae*	*Dendroctonus rufipennis*	*Dryocoetes confusus*
α-pinene	+	+	+	+
camphene	+	+	NR	NR
sabinene	+	+	+	NR
β-pinene	+	+	+	+
3-carene	+	+	+	+
limonene	+	+	+	+
β-phellandrene	+	+	+	+
myrcene	+	+	+	+
α-terpinene	NR	+	NR	+
p-cymene	+	+	+	NR
γ-terpinene	+	+	NR	+
terpinolene	+	+	+	+
bornyl acetate	+	NR	+	+

Table 3.2. Percent (–) enantiomer of chiral compounds in conifer bole and foliage. ND = not detected.

	Pseudotsuga menziesii		*Pinus contorta* var. *latifolia*		*Picea engelmannii* x *glauca*		*Abies lasiocarpa* x *bifolia*	
Compound	bole	foliage	bole	foliage	bole	foliage	bole	foliage
α-pinene	85.3	79.7	67.7	86.3	54.1	85.8	65.7	44.7
camphene	100	100	43.8	100	100	100	ND	100
sabinene	100	100	100	72.3	100	100	ND	ND
β-pinene	100	100	100	100	91.4	100	100	100
3-carene	0	0	0	0	0	0	0	0
limonene	55.5	57	38.9	77.7	68.3	0	100	84.9
β-phellandrene	100	100	100	100	100	100	100	100
bornyl acetate	100	100	100	100	ND	100	100	100

A. lasiocarpa x *bifolia,* and (+)- camphene and (+)-limonene predominated in the bole of *P. contorta* var. *latifolia*.

Antennal responses to volatiles from beetles

Figure 3.2. shows a representative chromatogram and antennal responses of both sexes of all four species to volatiles from unpaired female *D. pseudotsugae* excised from a Douglas-fir log. Nine compounds that elicited antennal responses were identified from volatiles of beetles (Table 3.3.). Of these, 1-octen-3-ol, *trans*-verbenol, verbenone and nonanal were found in all four species. 1-Octen-3-ol occurred exclusively in the volatiles of females. Frontalin and acetophenone were identified in the volatiles of *Dendroctonus* spp., with acetophenone occurring only in females.

3-Methyl-2-cyclohexen-1-one (MCH, also known as seudenone) was identified in volatiles of *D. pseudotsugae* and *D. rufipennis*. *exo*- and *endo*-Brevicomin were produced by *D. ponderosae* and *Dr. confusus*. All four species detected all of these compounds except for *Dr. confusus,* which did not detect frontalin. There were no qualitative (i.e. presence or absence of any compound) differences in the volatiles of beetles at different stages of attack within each sex and species. Chiral compounds in beetles were present in amounts that were too small to determine enantiomeric composition in this study, but have been identified in previous studies (Mayer & McLaughlin, 1991), except for 1-octen-3-ol, which occurred in the *R*- (–) form.

Figure 3.2. Representative responses (EAD) of both sexes of four species of tree-killing bark beetles to the volatiles of female *D. pseudotsugae* in log alone (FID). Identified FID peaks are (1) frontalin, (2) 1-octen-3-ol, (3) seudenone (MCH), (4) nonanal, (5) *trans*-verbenol, (6) verbenone.

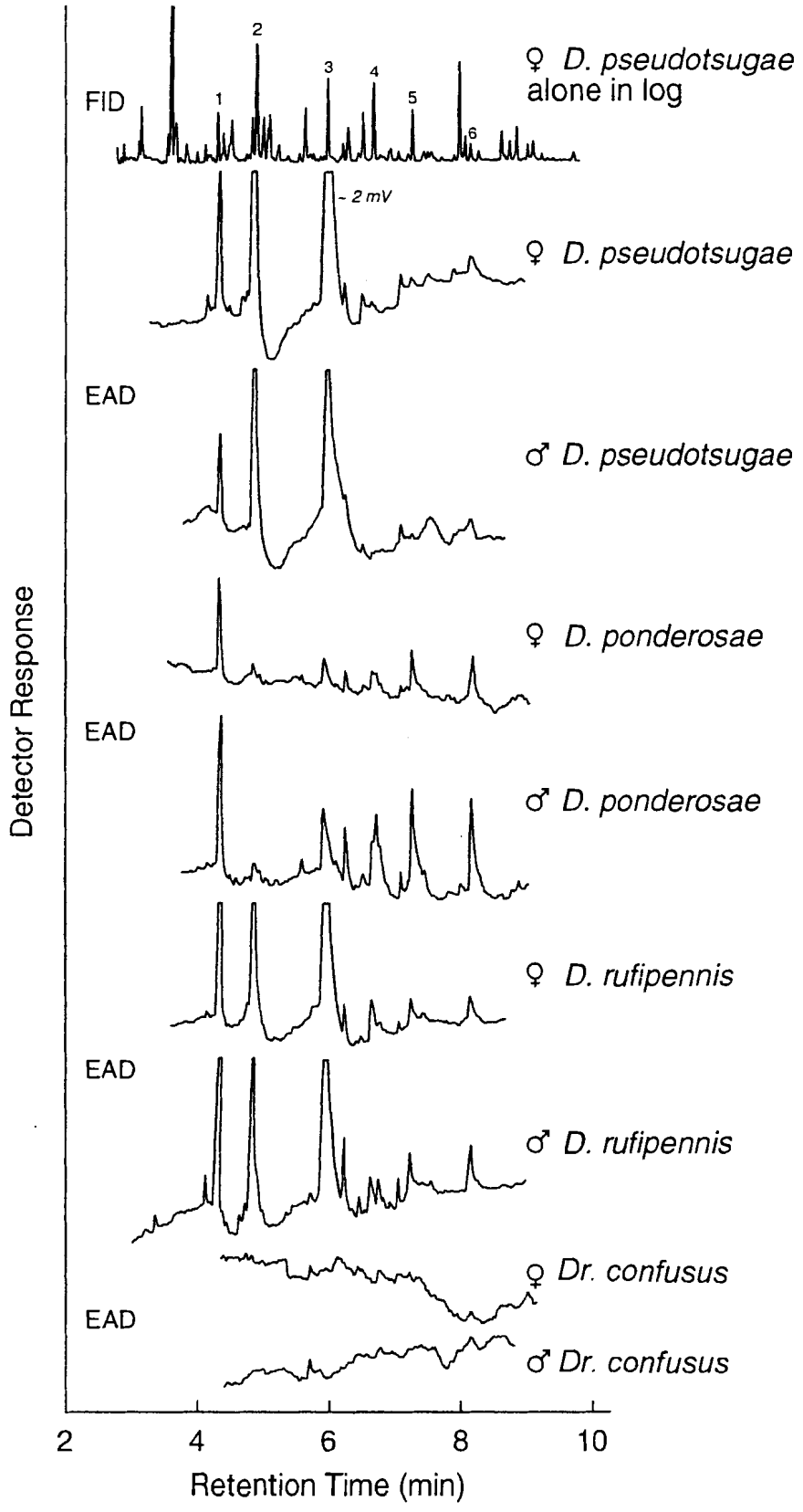

FID
1
2
3
4
5
6
♀ D. pseudotsugae
alone in log
~ 2 mV
♀ D. pseudotsugae
EAD
♂ D. pseudotsugae
♀ D. ponderosae
EAD
♂ D. ponderosae
♀ D. rufipennis
EAD
♂ D. rufipennis
♀ Dr. confusus
EAD
♂ Dr. confusus
Detector Response
2
4
6
8
10
Retention Time (min)

Table 3.3. Detection by four species of bark beetles of volatiles from sympatric con- and heterospecific beetles.

Compound	Species and sex in which compound was detected	Antennal response (+), or no response (NR)			
		Dendroctonus pseudotsugae	*Dendroctonus ponderosae*	*Dendroctonus rufipennis*	*Dryocoetes confusus*
frontalin	Both sexes of *D. pseudotsugae,* male *D. ponderosae* and both sexes of *D. rufipennis*	+	+	+	NR
1-octen-3-ol	Female *D. pseudotsugae, D. ponderosae, D. rufipennis* and *Dr. confusus*	+	+	+	+
seudenone	Both sexes of *D. pseudotsugae* and female *D. rufipennis*	+	+	+	+
trans-verbenol	Both sexes of *D. pseudotsugae, D. ponderosae* and male *Dr. confusus*	+	+	+	+

Compound	Species and sex in which compound was detected	Antennal response (+), or no response (NR)			
		Dendroctonus pseudotsugae	*Dendroctonus ponderosae*	*Dendroctonus rufipennis*	*Dryocoetes confusus*
verbenone	Both sexes of *D. pseudotsugae*, *D. ponderosae* and *D. rufipennis*	+	+	+	+
nonanal	Female *D. pseudotsugae* and both sexes of *D. ponderosae*, *D. rufipennis* and *Dr. confusus*	+	+	+	+
exo-brevicomin	Male *D. ponderosae* and both sexes of *Dr. confusus*	+	+	+	+
endo-brevicomin	Male *D. ponderosae* and both sexes of *Dr. confusus*	+	+	+	+
acetophenone	Female *D. pseudotsugae*, *D. ponderosae*, *D. rufipennis* and *Dr. confusus*	+	+	+	+

3.4. Discussion

Antennal responses to volatiles from conifers

All four species of tree-killing bark beetles exhibited antennal responses to the monoterpenes present in conifer volatiles. There were no qualitative differences between tree species, e.g. the occurrence of small amounts of species-specific minor components, or differences in the antennal response of the beetles to conifer volatiles that indicated host specificity or preference. In a subsequent study, there were many quantitative differences among conifer volatiles that could account for host specificity (Chapter 4).

Generally, monoterpenes synergise the effect of attractive pheromones to bark beetles (D.L. Wood, 1982; Borden, 1989). In a dose-dependent manner, 3-carene, myrcene and β-phellandrene increased the response to pheromones by *D. ponderosae*, and 3-carene, β-phellandrene and β-pinene increased the response to pheromones by *I. pini* (Miller & Borden, 2000). *Ips latidens* (LeConte) appeared to prefer β-pinene and β-phellandrene, over myrcene and terpinolene, which became less attractive with increasing doses (Miller & Borden, 2000). This capacity to respond to quantitative differences in monoterpenes suggests that coniferophagous bark beetles could use ratio-specific blends of monoterpenes to discriminate among potential host species that are qualitatively identical in monoterpene content.

Recognition of nonhosts may occur at short or long range. In Chapter 2, I found that landing by *Dr. confusus* was inhibited on nonhost interior spruce baited with the *Dr. confusus* pheromone, *exo*-brevicomin. In contrast, landing by female *D. rufipennis* was not inhibited on nonhost interior firs baited with pheromones. Moreover, attack was

attempted by *D. pseudotsugae* on pheromone-baited nonhost lodgepole pines and by *D. ponderosae* on nonhost Douglas-firs. Attack was not successful in either case, but the fact that beetles landed on nonhosts indicates that they were not repelled by volatiles from nonhost conifers. These results support previous suggestions (Hynum & Berryman, 1980; Moeck *et al.*, 1981; Raffa & Berryman, 1982b) that *Dendroctonus* spp. may not discriminate among conifers in flight and may have to land and test them for suitability.

Antennal response to volatiles from beetles

Eight compounds from volatiles of beetles elicited antennal responses from all four species. Although there are several shared components among two or more species, this result indicates that beetles have the potential to detect and respond behaviourally to compounds produced by other species that attack nonhost conifers. Only frontalin produced by the three *Dendroctonus* spp. did not elicit a response from *Dr. confusus*. Frontalin is an aggregation pheromone of *D. pseudotsugae* (Pitman & Vité, 1970) and *D. rufipennis* (Kline *et al.*, 1974; Furniss *et al.*, 1976) and is multifunctional in *D. ponderosae,* being attractive in very low amounts and repellent in high doses (Borden *et al.*, 1987a).

1-Octen-3-ol occurs in nature as the *R* -(–)-enantiomer (Pierce *et al.*, 1989; McMahon *et al.*, 2001) and was identified only in females of all four species. It is derived from the oxidation of linoleic acid (Tressl *et al.*, 1982) and is frequently found in fungal volatiles (Kaminski *et al.*, 1972). It is attractive to bont ticks (McMahon *et al.*, 2001), is an aggregation pheromone of the cucujid beetles *Oryzaephilus surinamensis* (L.) and *O.*

mercator (Fauvel) (Pierce *et al.,* 1989), and was identified in head space volatiles of the European bark beetle, *Xylocleptes bispinus* Duft, to which it was repellent (Klimetzek *et al.*, 1989). I hypothesise that it may also be repellent to bark beetles in this study.

Seudenone, (3-Methyl-2-cyclohexenone or MCH) is an antiaggregation pheromone of *D. pseudotsugae* (Rudinsky, 1973) and *D. rufipennis* (Kline *et al.*, 1974). It elicited antennal responses in *D. ponderosae* and *Dr. confusus* in whose volatiles it was not documented, suggesting that these two species could potentially use it to detect and avoid a nonhost conifer under attack by heterospecific beetles. *trans*-Verbenol, a potent aggregation pheromone of *D. ponderosae* (Pitman & Vité, 1969), was also detected in the volatiles of *D. pseudotsugae* and *D. rufipennis*. *trans*-Verbenol has been previously identified in the volatiles of *D. pseudotsugae* and was found to be attractive in low concentrations and repellent at high concentrations (Rudinsky *et al.*, 1972). A strong *trans*-verbenol signal may indicate a tree under attack by *D. ponderosae* and there may be a dose-dependent effect of *trans*-verbenol on the other species, with high doses indicating the presence of an unsuitable host.

Verbenone, an antiaggregation pheromone of *D. ponderosae* (Ryker & Yandell, 1983; Borden *et al.*, 1987a), was identified in the three *Dendroctonus* species. It has been identified to be antiaggregative in other *Dendroctonus* spp. including *D. frontalis* Zimmerman, *D. brevicomis* LeConte and *D. adjunctus* Bland (Renwick, 1967; Renwick & Vité, 1970; Payne *et al.*, 1978; Livingston *et al.*, 1983), and would indicate a tree that is at an advanced stage of attack.

Nonanal is found in volatiles of all four species of beetles, in volatiles of lodgepole pine (Pureswaran *et al.*, 2000), and in angiosperm bark (Huber *et al.*, 2000). It decreases the responses of *D. pseudotsugae* (Huber & Borden, 2001a), *D. ponderosae* (Borden *et al.*, 1998) and *I. pini* (Pureswaran *et al.* 2000) to their aggregation pheromones.

exo-Brevicomin is a multifunctional pheromone produced by male *D. ponderosae* that is attractive in low concentrations and repellent at high concentrations (Rudinsky *et al.*, 1974a). *exo*- and *endo*-Brevicomin are also aggregation pheromones produced by male *Dr. confusus* (Borden *et al.*, 1987b). They were not detected in the volatiles of the other two *Dendroctonus* spp., even though they exhibited an antennal response to them, indicating that these compounds could be used by other the species as cues to avoid nonhosts.

Acetophenone was found in the volatiles of females of all four species. When tested against *D. ponderosae*, it did not invoke a behavioural response (Pureswaran *et al.* 2000). Its biological activity in the other three species in this study is not known.

Sympatric species colonising the same host are characteristically repelled by pheromones of heterospecifics on the same tree, aiding in resource partitioning (Byers & Wood, 1980; Borden *et al.*, 1992; Poland & Borden, 1998a,b,c; Savoie *et al.*, 1998). My finding of antennal responses to a wide range of pheromones of heterospecific beetles that attack nonhost conifers indicates that they can potentially be used as cues to avoid the wrong host.

4. Quantitative variation in monoterpenes among four species of conifers

4.1. Introduction

In Chapter 3, I found that monoterpene profiles of the four sympatric species of conifers were not qualitatively different (there were no species-specific major or minor components), and their respective herbivores, *D. pseudotsugae*, *D. ponderosae*, *D. rufipennis*, and *Dr. confusus*, did not differ in their antennal responses to monoterpenes in these trees. Although monoterpenes were qualitatively similar among species, I hypothesised that there may be quantitative variation in monoterpene composition among sympatric species of conifers. Beetles could potentially use this information to discriminate among host and nonhost conifers. So, I used analytical chemistry techniques to extract and analyse the monoterpene composition of the four species of conifers.

Volatiles released from either bole tissue or foliage could potentially be used by beetles to orient toward or away from conifers. Studies on chemical composition of conifers, including the four species in this study, have focussed on monoterpene composition of either foliage monoterpenes (Ogilvie & von Rudloff, 1968; von Rudloff, 1972a,b, 1975; Forrest, 1980) or xylem oleoresin (Mirov, 1961; Zavarin & Cobb, 1970; Zavarin *et al.*, 1970; Smith, 1983, 2000). While foliar volatiles are ubiquitous in a forest environment, dispersing bark beetles may also encounter the combined bole volatiles from both bark and xylem, after trees are mechanically damaged, e.g. through lightning,

frost cracks or wind breakage, or after penetration of phloem and scoring of xylem by pioneer beetles (Rudinsky, 1962; Seybold *et al.*, 2000). While monoterpenes in bark and xylem oleoresin may vary quantitatively (Tomlin *et al.*, 2000), resin ducts that contain high amounts of monoterpenes in pines and spruces may be interconnected and pass from the xylem into the phloem (Chang, 1954; Lewinsohn *et al.*, 1991). Accordingly, Lewinsohn *et al.* (1991) used extracts of whole stems of conifer saplings to estimate monoterpene content and extractable monoterpene cyclase activity in a range of conifer species.

Chiral specificity by bark beetles to host monoterpenes (Hobson *et al.*, 1993) as well as to pheromones (Miller *et al.*, 1989; Seybold *et al.*, 1995) has been documented, particularly when monoterpenes serve as pheromone precursors (Renwick *et al.*, 1976a; Klimetzek & Francke, 1980). The ability of *I. paraconfusus* and *Ips typographus* L. to produce their aggregation pheromone *cis*-verbenol is determined by (-)-α-pinene in the host, as its antipode is converted to *trans*-verbenol (Renwick *et al.*, 1976b; Klimetzek & Francke, 1980). In addition, while a mixture of (-)-α-pinene and (+)-limonene attracted *I. typographus* to pheromone baited traps, (+)-α-pinene repelled them (Reddemann & Reinhard, 1996). The percentage of aggregation pheromone (-)-ipsdienol produced by male *I. pini*, declined on other conifers compared to the host of origin (Seybold *et al.*, 1995).

Studies on European conifers have established enantiomeric composition of monoterpenes (Persson *et al.*, 1996; Sjodin *et al.*, 1996, 2000), whereas except for Hobson *et al.*,'s (1993) study of monoterpenes in *P. ponderosa*, most North American

studies (Mirov 1961; von Rudloff, 1972a,b, 1975; Smith 1983, 2000) have not documented enantiomeric composition, nor analysed monoterpene compositions using statistical procedures beyond that of normalised percent composition, and measures of central tendency. There is therefore, a lack of information on precise quantitative differences in monoterpene amounts among conifer species.

Variation in the relative amounts of monoterpenes in conifers have been observed among: 1) tissues of the same tree (Persson *et al.*, 1993, 1996; Sjodin *et al.*, 1996, 2000), 2) trees within a population (Zavarin *et al.*, 1990; Sadof & Grant, 1997), 3) populations within a species (Zavarin *et al.*, 1990; Smith, 2000), and 4) among different species (von Rudloff, 1975; Smith, 2000). I report in this chapter, the intra- and interspecific quantitative variation of 18 monoterpenes identified in the bole tissues (bark and sapwood) and foliage of four sympatric conifers, in three locations (four for Douglas-fir) in British Columbia (B.C.). I document enantiomeric composition, compare monoterpene amounts between bole tissues and foliage, and use multiple comparisons and principal components analyses to determine intraspecific variation in monoterpene composition among three sites in B.C., and interspecific variation across the four species. All 18 monoterpenes are perceived by the antennae of all of the above four species of bark beetles (Chapter 3).

4.2. Materials and Methods

Collection of samples

Samples of bole and foliage from each species were collected in late August 2000, from three locations, Princeton, 100 Mile House and Prince George in the interior of B.C. for all four species. The three locations are approximately equidistant along a 475 km south to north transect. Douglas-fir was also sampled at a fourth site at Maple Ridge, 150 km west of Princeton, in the lower Fraser Valley on the Pacific coast. Ten trees from each species that were $\geq$ 25 cm in diameter at a height of 1.3 m, and were at least 500 m apart were sampled in each site. For each tree, a sample of bole tissue including outer bark, phloem, and sapwood was removed using a sharp hatchet and collected in a glass jar. A branch of foliage from the same tree at a height of about 3.5 m was clipped using a pole-pruner, and placed in a plastic bag. Samples were stored over dry ice for transport to the laboratory, and then stored at -15°C until they were extracted and analysed.

Extraction and analysis of monoterpenes

Bole and foliage samples were dipped in liquid nitrogen to arrest metabolic activity. Needles from current and two previous years' growth were pooled and ground in liquid nitrogen using a coffee grinder. Bole samples (outer bark, phloem and sapwood) were chopped with a knife and similarly ground. A 1.5 g ground subsample of each tissue, was set aside for determination of dry weight, and the same amount was extracted in 8 ml of EtOH: MeOH: H_2O (79:20:1) to which heptyl acetate was added as an internal standard, using a hand-held homogeniser (Tomlin *et al.*, 1997). Chlorophyll and resin acids were

removed from extracts by successive filtrations through activated charcoal and cotton, and DEAE Sephadex in the basic form (Zinkel & Magee, 1991). The filtrate was extracted with hexane, the monoterpene fraction was dried over magnesium sulphate, and amounts of 18 monoterpenes were quantified by gas chromatography (GC). A Hewlett Packard Model 5890 equipped with a fused silica column (DB-5, 30 m x 0.32 mm ID, J&W Scientific, Folsom, CA 95630) was used with a temperature programme of 50°C to 200°C, at 10°C per min with split injections. Helium was the carrier gas with a flow rate of 1 cubic cm per min.

The enantiomeric composition of optically active compounds were determined using a cyclodex-B column (30 m x 0.25 mm ID, J&W Scientific, Folsom CA 95630-4714) with a temperature programme of 80°C held for 12 min, increased at 5°C / min. to 150°C, held for 5.5 min, then cooled at 5°C / min. to 80°C. Compounds were identified by comparison of retention times and indices (van den Dool & Kratz, 1963) with a mixture of synthetic standards by GC-MS (Varian Saturn Ion Trap). The temperature programme was 50°C for 1 min and then 10°C / min to 280°C. Injector and detector ports were held at 250°C and 260°C, respectively.

Statistical analyses

Data were transformed by $\log_{10}(x+1)$ to satisfy assumptions of normality and homoscedasticity. Differences in mean monoterpene amounts between bole and foliage tissues of a species within a site were compared using t-tests (Proc T-TEST) (SAS Institute Inc., 1990). Sequential Bonferroni adjustments were applied to account for the

number of tests that were performed (Rice, 1989). Means of total extractable amounts of monoterpenes from bole and foliage of each species were compared using analysis of variance (Proc ANOVA), with species as the source of variation, followed by the REGW multiple range test (Day & Quinn, 1989).

Multivariate analysis of variance (Proc ANOVA) and REGW multiple range tests (SAS Institute Inc., 1990) were performed to determine differences in mean amounts of monoterpenes in bole and foliage: 1) between sites within a species, with site as the main effect and 2) among the four species, with species, site and their interaction included in the model. The species term was tested against the species*site mean square. REGW tests correct for Type I experiment-wise error, to account for the number of comparisons performed (Day and Quinn, 1989; SAS Institute Inc., 1990).

Principal components analysis (Proc PRINCOMP, SAS Institute Inc. 1990) was used to summarise relationships among several quantitative variables, to summarise multivariate data in two dimensions, and discern if clustering of species, or sites within a species occurred based on total complement of monoterpenes. Correlation coefficients (Proc CORR) between the monoterpene amounts, and the first two principal components were calculated to determine which monoterpenes were responsible for the separation of the overall monoterpene profiles among species, and among sites for Douglas-fir.

4.3. Results and Discussion

Variation in total extractable monoterpenes among species

There was significant variation in the total amount of all extractable monoterpenes among the four species of conifers (Figure 4.1) (bole: $F = 166.99$, $df = 3, 2156$, $P < 0.0001$; foliage: $F = 212.67$, $df = 3, 2156$, $P < 0.0001$). Interior fir had the highest extractable amount of bole and foliage volatiles, being over five and two times greater respectively, than the other species.

Variation among trees and between bole tissues and foliage

Compared to bole tissues, extractable monoterpenes from foliage were nine times greater in interior Douglas-fir, one third in lodgepole pine, half in interior spruce, and not significantly different in interior fir (Figure 4.1). Variation in total monoterpene composition among tissues is common in conifers (von Rudloff, 1972a, 1975). For example, monoterpene concentration in foliage of Scots pine *Pinus sylvestris* L., was five times greater than that of wood (Manninen et al., 2002).

Although total extractable monoterpenes did not vary greatly among the 30 trees sampled (Figure 4.1.), the amounts of individual monoterpenes in all four species were highly variable (Table 4.1.). In most of the monoterpenes listed in Table 4.1., the coefficient of variation (CV = standard deviation/mean) of monoterpenes that constitute at least 5% was > 75%. The least and most variable compounds were (-)-α-pinene (CV = 36.76%) and (-)-β-pinene (CV = 515.37 %) in bole and foliage, respectively, of coastal

Figure 4.1. Variation in the mean amount of extractable monoterpenes in pooled bole and foliage samples of interior Douglas-fir, *Pseudotsuga menziesii*, lodgepole pine, *Pinus contorta* var. *latifolia*, interior spruce *Picea engelmannii* x *glauca*, and interior fir, *Abies lasiocarpa* x *bifolia*. Comparisons are among species. Bars within a subgraph with different letters are significantly different, REGW multiple comparisons test, $P < 0.05$. N = 30 for each species.

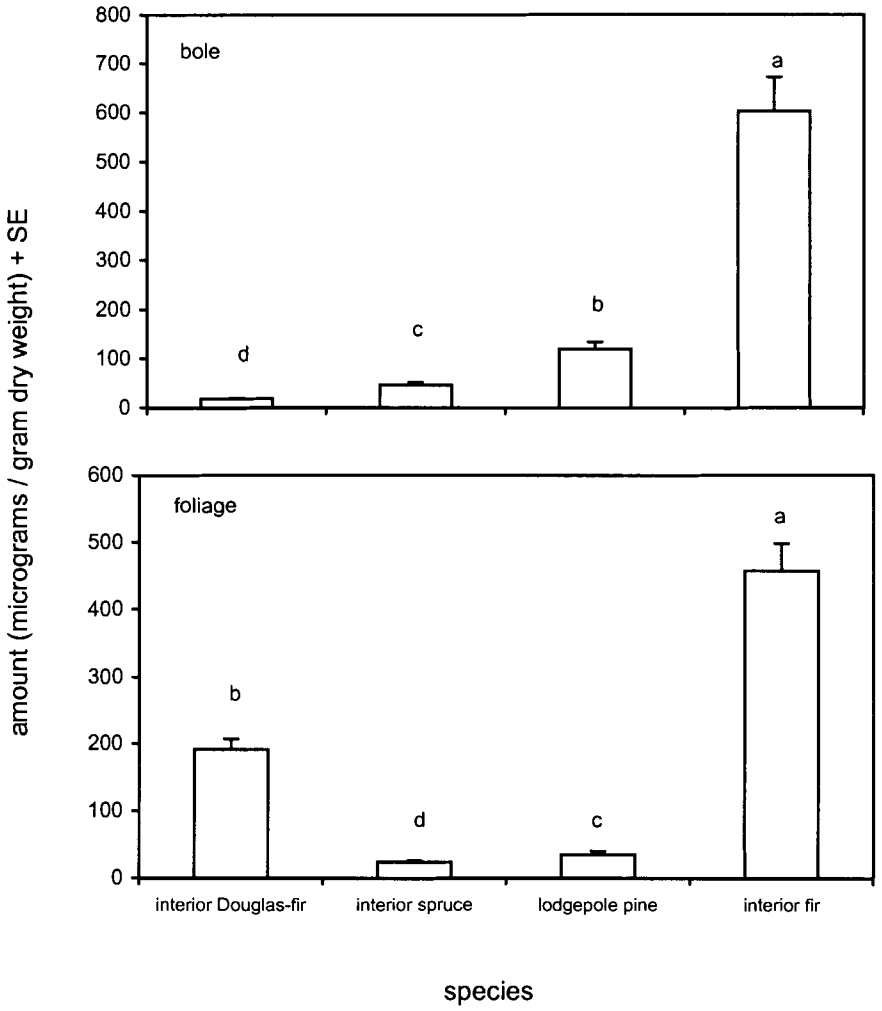

bole
foliage
amount (micrograms / gram dry weight) + SE
species
interior Douglas-fir
interior spruce
lodgepole pine
interior fir
a
b
c
d

Table 4.1. Percent composition of monoterpenes that constitute ≥ 5% of total volatiles and their coefficient of variation (CV)

Species	N	Tissue	Monoterpene	Mean Percent composition	CV %
coastal Douglas-fir	10	bole	(-)-α-pinene	63.80	36.76
			(-)-camphene	11.95	60.03
			(-)-sabinene	5.15	68.98
			(-)-β-pinene	6.86	75.54
			others	12.24	
		foliage	(-)-α-pinene	10.7	114.13
			(-)-sabinene	31.2	418.57
			(-)-β-pinene	39.7	515.37
			terpinolene	11.5	137.95
			others	6.9	
interior Douglas-fir	30	bole	(-)-α-pinene	30.83	67.30
			(+)-α-pinene	29.98	90.36
			(-)-β-pinene	21.36	78.00
			others	17.86	
		foliage	(-)-α-pinene	15.4	49.69
			(-)-camphene	25.3	56.60
			(-)-sabinene	6.4	153.48
			(-)-β-pinene	15.6	69.38
			(-)-bornyl acetate	26.1	60.07
			others	11.2	
lodgepole pine	30	bole	(-)-α-pinene	4.9	79.26
			(-)-β-pinene	15.8	101.64
			(+)-3-carene	5.76	150.67
			(-)-β-phellandrene	50.1	79.2
			(-)-limonene	5.93	170.32
			others	17.51	

Species	N	Tissue	Monoterpene	Mean Percent composition	CV %
		foliage	(-)-α-pinene	7.7	108.82
			(-)-β-pinene	47.0	125.97
			(-)-β-phellandrene	36.9	103.76
			others	8.4	
interior spruce	30	bole	(-)-α-pinene	29.9	76.20
			(+)-α-pinene	22.9	81.56
			(-)-β-pinene	23.2	88.79
			(+)-3-carene	8.82	211.58
			(-)-β-phellandrene	5.76	171.23
			others	9.42	
		foliage	(-)-α-pinene	7.9	121.44
			(+)-α-pinene	6.8	138.60
			(-)-camphene	9.7	151.93
			(+)-camphene	11.5	156.47
			myrcene	15.6	163.98
			limonene	8.3	113.74
			terpinolene	6.6	129.08
			(-)-bornyl acetate	19.8	122.66
			others	13.8	
interior fir	30	bole	(-)-α-pinene	9.6	95.48
			(-)-β-pinene	25.3	65.33
			(+)-3-carene	9.57	68.75
			(-)-β-phellandrene	40.8	91.72
			(-)-limonene	8.9	122.93
			others	5.83	
		foliage	(-)-α-pinene	4.9	40.00
			(-)-camphene	8.6	110.95
			(-)-β-pinene	17.52	79.27
			(-)-β-phellandrene	32.95	61.91
			(-)-limonene	13.57	75.21
			(-)-bornyl acetate	15.71	83.52
			others	6.75	

Douglas-fir. Variation in monoterpene amounts between trees in a population is common in conifers, and has been recorded in *Pinus pinaster* Ait. (Jactel *et al.*, 1996), ponderosa pine, *Pinus ponderosa* Laws. (Latta *et al.*, 2000), Norway spruce, *Picea abies* L. (Persson *et al.*, 1996), western white pine, *Pinus monticola* Dougl. (Zavarin *et al.*, 1990), the central American pine, *Pinus oocarpa* Schiede (Lockhart, 1990), and larch, *Larix* spp. (Holm & Hiltunen, 1997).

In my lodgepole pine samples from Prince George, there were significant differences between amounts in bole and foliage for 18 compounds analysed (Table 4.2.). In contrast, among interior spruce trees that I sampled from Prince George, and interior firs sampled from Princeton and Prince George, there were differences only in five of the 18 compounds. Amounts of (-)-camphene, terpinolene and (-)-bornyl acetate were significantly different between bole and foliage in 10 of the 13 sets of trees sampled, while amounts of (+)-sabinene and α-terpinene were different only in three cases. Differences in monoterpene profiles among tissues of the same tree are also common in conifers. Monoterpene profiles of cone and foliage volatiles of Swiss stone pine, *Pinus cembra* L., were also significantly different (Dormont *et al.*, 1998). In *P. ponderosa*, wood, roots and exuded xylem oleoresin contained high amounts of α-pinene, while foliage contained high amounts of β-pinene (Latta *et al.*, 2000). There were greater differences in monoterpene amounts between foliage and xylem of *P. abies* than within each tissue (Persson *et al.*, 1996). In *P. sylvestris*, monoterpene composition was similar between branch and bole xylem, but differed between xylem and foliage (Sjodin *et al.*, 2000).

Table 4.2. Differences in monoterpene amounts between bole and foliage in four species of conifers in three or four different sites as expressed by t-tests. Sequential Bonferroni adjusted $\alpha \leq 0.05$. Asterisks indicate significant difference.

		Douglas-fir				lodgepole pine			interior spruce			interior fir		
Monoterpene		Maple Ridge	Princeton	100 Mile House	Prince George	Princeton	100 Mile House	Prince George	Princeton	100 Mile House	Prince George	Princeton	100 Mile House	Prince George
(-)-α-pinene	t	-0.47	8.85	8.35	6.13	0.57	-1.95	-3.85	-3.95	-5.34	-5.28	-2.17	-4.77	-2.50
	P	0.6468	<0.0001*	<0.0001*	<0.0001*	0.5769	0.0672	0.0012*	0.0009*	<0.0001*	<0.0001*	0.0437	0.0002*	0.0223
(+)-α-pinene	t	1.02	1.66	-4.19	-2.19	-1.51	-4.32	-5.48	-4.23	-4.80	-4.17	-2.63	-3.36	-1.88
	P	0.3198	0.1142	0.0006*	0.0422	0.1495	0.0004*	<0.0001*	0.0005*	0.0001*	0.0006*	0.0171	0.0035*	0.0761
(-)-camphene	t	-5.63	13.33	33.88	12.64	-10.25	-14.12	-8.74	4.14	0.33	1.56	5.45	7.40	1.75
	P	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0006*	0.7422	0.1355	<0.0001*	<0.0001*	0.0965
(+)-camphene	t	-2.2	1.01	1.66	0.58	-6.85	-5.58	-8.04	5.80	2.77	5.25	5.63	6.28	-2.22
	P	*P* = 0.0409	0.3258	0.1148	0.5687	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0125	<0.0001*	<0.0001*	<0.0001*	0.0392
(-)-sabinene	t	6.3	3.21	5.59	3.17	-2.72	-2.07	-3.47	-1.34	-1.83	-1.00	-1.69	-5.76	-0.50
	P	<0.0001 *	0.0048*	<0.0001*	0.0053*	0.0142	0.0531	0.0027*	0.1965	0.0841	0.3306	0.1091	<0.0001*	0.6240
(+)-sabinene	t	0.16	2.34	1.96	2.63	-4.23	-3.85	-3.30	0.16	0.97	1.48	-0.66	-2.44	-1.65
	P	0.8755	0.0308	0.0658	0.01	0.0005*	0.0012*	0.0040*	0.8755	0.3440	0.1572	0.5167	0.0254	0.1165
(-)-β-pinene	t	8.44	15.54	6.89	2.22	-2.09	-0.02	-2.36	-5.53	-7.81	-7.91	-0.98	-2.62	-1.79
	P	<0.0001*	<0.0001*	<0.0001*	0.0395	0.0510	0.9830	0.0298*	<0.0001*	<0.0001*	<0.0001*	0.3410	0.0174	0.0910
(+)-β-pinene	t	4.7	5.25	1.26	0.03	-1.46	-0.57	-4.07	-1.00	-2.11	-0.51	8.02	5.80	0.81
	P	0.0002*	<0.0001*	0.2232	0.9766	0.1610	0.5760	0.0007*	0.3325	0.0488	0.6183	<0.0001*	<0.0001*	0.4296
myrcene	t	15.52	18.50	3.33	2.42	-6.87	-5.48	-6.14	1.36	0.02	0.63	0.84	-0.69	1.16
	P	<0.0001*	<0.0001*	0.0037*	0.0264	<0.0001*	<0.0001*	<0.0001*	0.1921	0.9841	0.5335	0.4100	0.5006	0.2613
(+)- 3-carene	t	4.74	2.01	-0.07	4.68	-4.40	-2.47	-3.92	-0.84	-0.93	-1.91	-9.32	-14.91	-7.08
	P	0.0002*	0.0592	0.9451	0.0002*	0.0003*	0.0240	0.0010*	0.4117	0.3658	0.0723	<0.0001*	<0.0001*	<0.0001*
α-terpinene	t	1.23				-16.25	-2.95	-4.51	-1.00	-1.00	-1.00	-2.34	-23.89	-0.98
	P	0.2239				<0.0001*	0.0085	0.0003*	0.3306	0.3306	0.3306	0.0311	<0.0001*	0.3412
p-cymene	t	1.25	2.79	0.65	-1.00	-15.86	-18.37	-40.35	2.62	1.76	2.76	-2.02	-0.03	-4.58
	P	0.2284	0.0122	0.5237	0.3306	<0.0001*	<0.0001*	<0.0001*	0.0173	0.0953	0.0129	0.0580	0.9744	0.0002*
(-)-β	t	5.0	7.36	3.47	5.81	-4.15	-2.96	-3.43	-0.98	-4.54	1.62	-0.91	-2.48	0.91
phellandrene	*P*	< 0.0001*	<0.0001*	0.0027*	<0.0001*	0.0006*	0.0084	0.0030*	0.3403	0.0003*	0.1236	0.3734	0.0232	0.3774
(-)-limonene	t	1.07	9.46	9.69	10.60	-5.53	-4.95	-6.57	1.88	1.95	0.52	1.10	-0.41	5.13
	P	0.3006	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0001*	<0.0001*	0.0764	0.0675	0.6104	0.2846	0.6892	<0.0001*
(+)-limonene	t	-0.95	8.02	9.75	7.90	-8.36	-5.38	-7.82	0.32	0.28	2.47	2.83	3.93	6.00
	P	0.3554	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.7539	0.7840	0.0236	0.0112	0.0010*	<0.0001*
γ-terpinene	t	10.19	6.71	5.28	5.09	-4.91	-2.42	-3.44	-1.47	4.37	-1.00	0.72	-1.63	0.34
	P	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0261	0.0029*	0.1575	0.0004*	0.3306	0.4806	0.1205	0.7364
terpinolene	T	10.35	10.15	12.51	10.84	-6.78	-3.85	-7.52	4.25	3.60	2.33	-0.87	-3.27	1.18
	P	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0012*	<0.0001*	0.0005*	0.0020*	0.0315	0.3935	0.0043*	0.2551
(-)-bornyl	t	2.47	15.10	13.54	17.00	-4.07	-1.12	-4.39	3.26	4.10	8.35	7.22	12.06	6.39
acetate	*P*	0.0237	<0.0001*	<0.0001*	<0.0001*	0.0007*	0.2793	0.0004*	0.0043	0.0007*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Geographic variation in Douglas-fir

Multivariate analysis of variance revealed differences in the total monoterpene profiles among trees in four sites (bole: Wilks' $\lambda = 0.0021$, $F = 8.15$, $df = 51, 60.35$, $P < 0.0001$; foliage: Wilks' $\lambda = 0.0049$, $F = 5.29$, $df = 54, 57.43$, $P < 0.0001$). The major component of coastal Douglas-fir bole volatiles was (-)-α-pinene, while trees in the interior had both enantiomers of α-pinene as well as (-)-β-pinene predominating (Figure 4.2.). Trees from Princeton, the most arid site, had much smaller amounts of bole volatiles than trees in the other three locations. The foliage profile of coastal Douglas-fir was characterised by high amounts of (-)-β-pinene and (-)-sabinene, while (-)-camphene and (-)-bornyl acetate predominated in the interior (Figure 4.3.). The amounts of α-pinene, camphene, myrcene, limonene and (-)-bornyl acetate were significantly lower, and α-terpinene and *p*-cymene were significantly higher in foliage of coastal trees in Maple Ridge compared to trees in the three interior sites in the interior (Figure 4.3.). My results are consistent with von Rudloff's (1972a, 1973a,b) non-statistical analyses of Douglas-fir leaf oil. Because these intraspecific differences are greater than interspecific differences among spruces, von Rudloff (1972a) proposed re-classifying coastal and interior Douglas-fir as different chemotaxonomic species rather than as varieties. However, limited chemical sampling of tissues, such as foliage alone, is not sufficient to draw taxonomic or evolutionary conclusions.

In support of von Rudloff's (1972a, 1973a,b) studies, principal components analysis revealed a distinct separation of Douglas-firs in Maple Ridge from those in the

Figure 4.2. Quantitative variation in bole monoterpenes of Douglas-fir, *Pseudotsuga menziesii*, across four sites in British Columbia. Bars with different letters indicate significant differences in mean amounts of compounds across sites, REGW multiple comparisons test, $P < 0.05$, N = 10 per site. Bars with no letter indicate no difference across sites. Note variable scales on Y-axes.

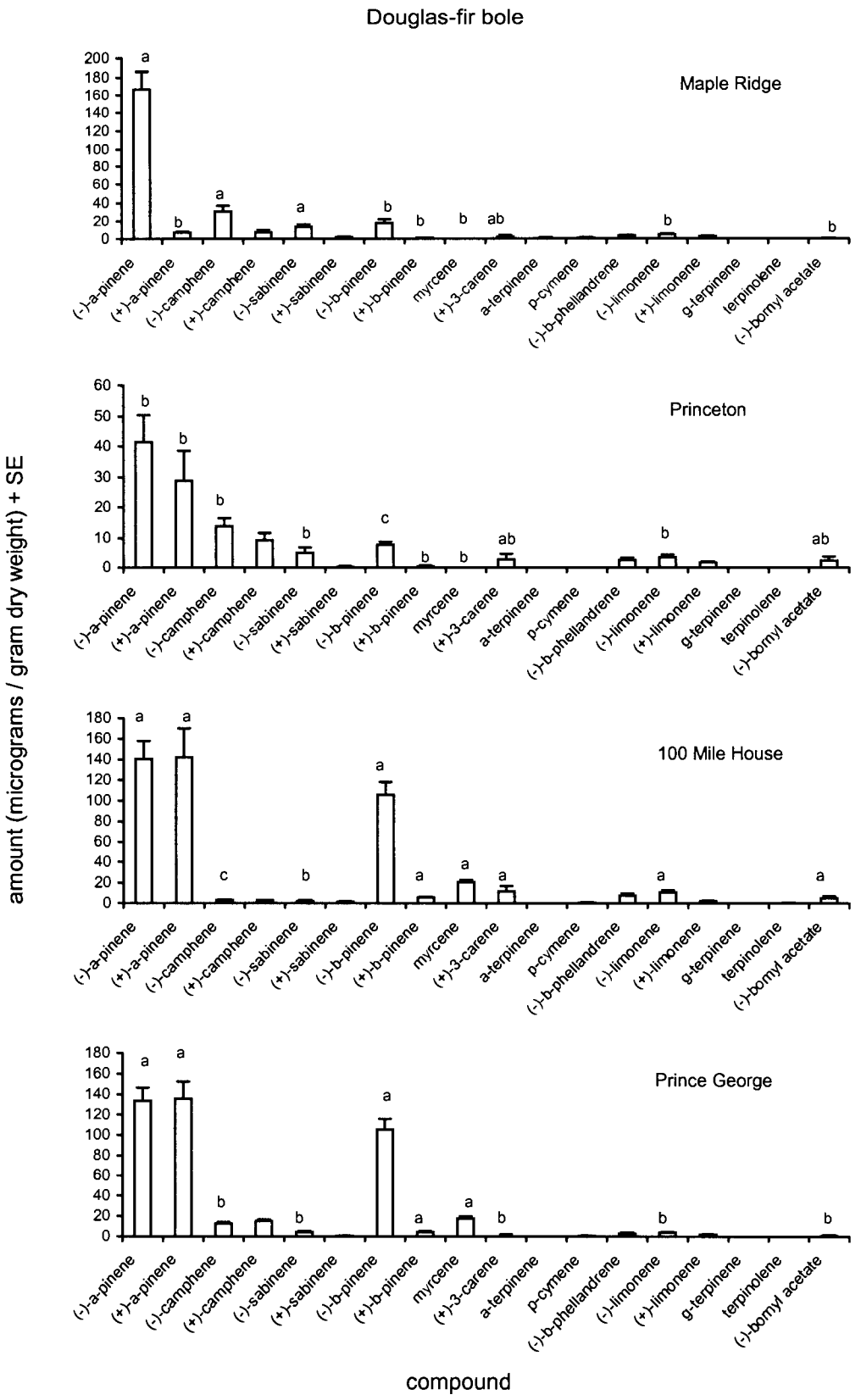
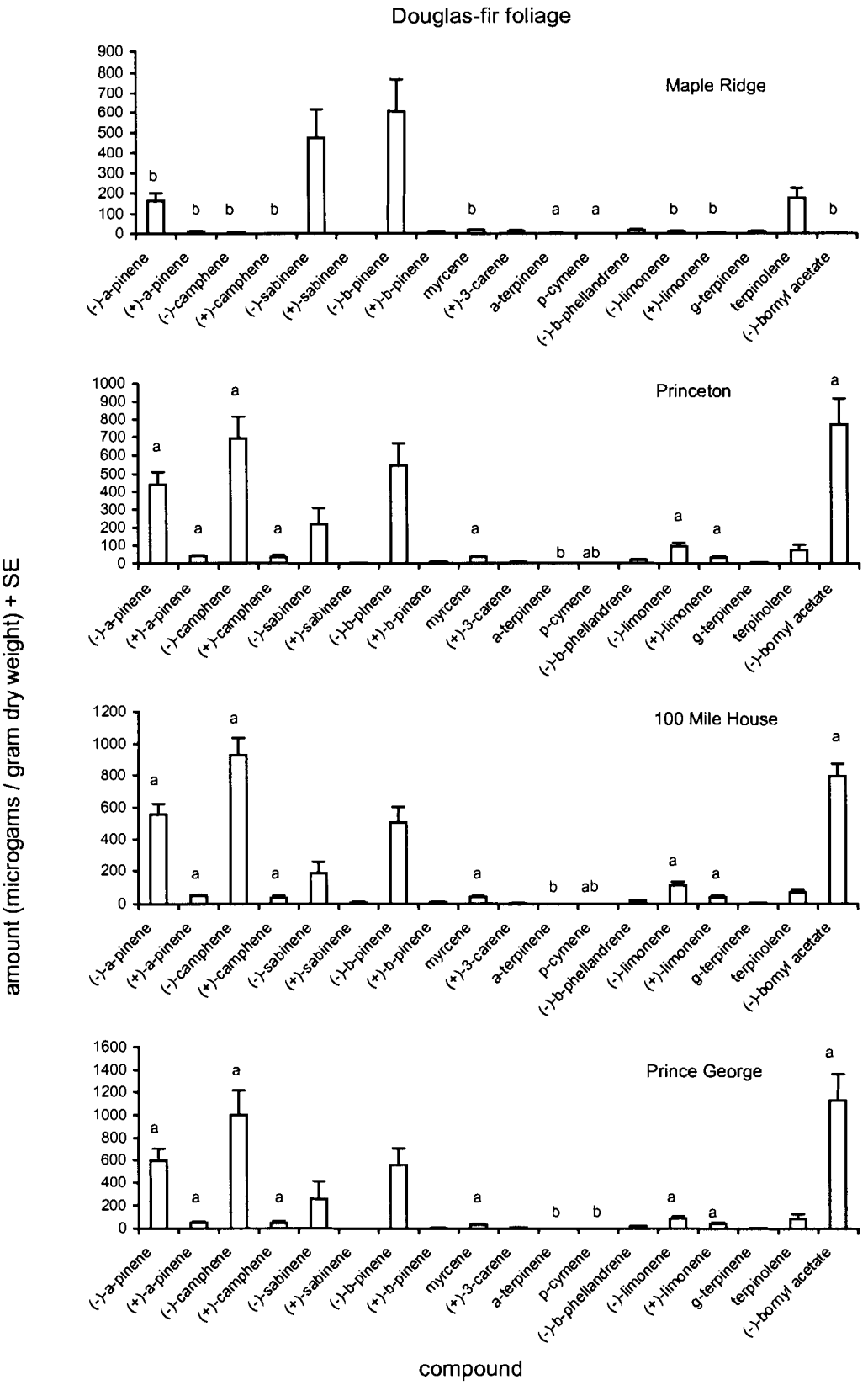

Douglas-fir bole
Maple Ridge
Princeton
100 Mile House
Prince George
amount (micrograms / gram dry weight) + SE
compound
(-)-a-pinene
(+)-a-pinene
(-)-camphene
(+)-camphene
(-)-sabinene
(+)-sabinene
(-)-b-pinene
(+)-b-pinene
myrcene
(+)-3-carene
a-terpinene
p-cymene
(-)-b-phellandrene
(-)-limonene
(+)-limonene
g-terpinene
terpinolene
(-)-bornyl acetate

Figure 4.3. Quantitative variation in foliage monoterpenes of Douglas-fir, *Pseudotsuga menziesii*, across four sites in British Columbia. Bars with different letters indicate significant differences in mean amounts of compounds across sites, REGW multiple comparisons test, $P < 0.05$, N = 10 per site. Bars with no letter indicate no difference across sites. Note variable scales on Y-axes.


Douglas-fir foliage
Maple Ridge
Princeton
100 Mile House
Prince George
amount (microgams / gram dry weight) + SE
compound
(-)-a-pinene
(+)-a-pinene
(-)-camphene
(+)-camphene
(-)-sabinene
(+)-sabinene
(-)-b-pinene
(+)-b-pinene
myrcene
(+)-3-carene
a-terpinene
p-cymene
(-)-b-phellandrene
(-)-limonene
(+)-limonene
g-terpinene
terpinolene
(-)-bornyl acetate

other three locations (Figure 4.4.), although a lack of complete separation in bole volatiles between trees from Maple Ridge and Princeton suggests that the Maple Ridge population is more closely related to trees from Princeton than to trees in the more northern interior locations.

Correlation analyses of monoterpene amounts with the first two principal components are reported in Table 4.3. PC 1 distinguishes populations with high amounts of (+)-α-pinene, (-)-β-pinene, (+)-β-pinene, and myrcene, low amounts of (-)-camphene, (-)-sabinene in bole volatiles, i.e. 100 Mile House and Prince George, from populations with the reverse relative amounts of those volatiles, i.e. Maple Ridge and Princeton. PC 2 separates out trees with high camphene and sabinene, and low 3-carene, but trees with those monoterpenes were found in all populations. In foliage, PC 1 separates out the population from Maple Ridge that has low relative amounts of (+)-α-pinene, (-)-α-pinene, (-)-camphene, (+)-camphene, myrcene, (-)-limonene, (+)-limonene, and (-)-bornyl acetate compared to trees in the three interior sites. PC 2 separates out trees with high amounts of (-)-sabinene, myrcene, (+)-3-carene, (-)-β-phellandrene, γ-terpinene, and terpinolene. As with bole volatiles, trees with these monoterpenes in foliage, are found in all four populations.

Geographic variation in other species

There were only minor differences in bole volatiles, and none in foliage volatiles of lodgepole pine among the three interior sites (Figures 4.5., 4.6.). Mirov (1961) and Smith (1983, 2000) reported β-phellandrene as the main constituent of lodgepole pine resin,

Figure 4.4. Principal components analysis of entire complement of bole and foliage monoterpenes of Douglas-fir, *Pseudotsuga menziesii*, in four sites in British Columbia. PC1 and PC2 accounted for 41.5 % and 13.6 % of variation, respectively in bole volatiles and 59.2 % for 19.3 %, respectively, in foliage volatiles. Polygons are drawn around identical numbers to aid in showing separation or overlap among populations.

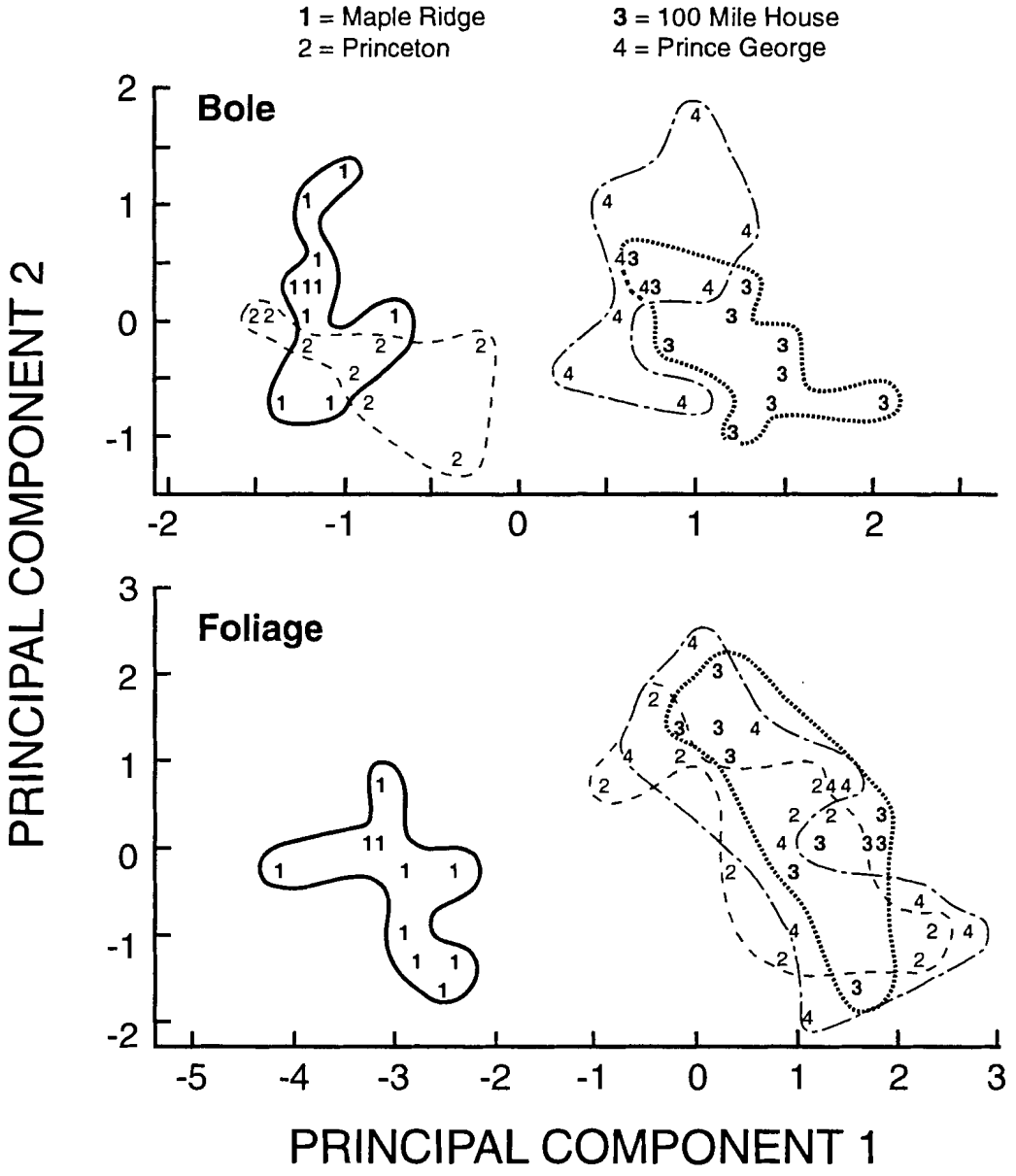

1 = Maple Ridge
2 = Princeton
3 = 100 Mile House
4 = Prince George
Bole
Foliage
PRINCIPAL COMPONENT 2
PRINCIPAL COMPONENT 1

Table 4.3. Eigenvectors, correlation coefficients (r), and *P*-values of monoterpenes in bole and foliage of Douglas-fir with the first two principal components. Sequential Bonferroni adjusted $\alpha \leq 0.05$. Asterisks indicate significance.

		Principal component 1			Principal component 2		
Tissue	Monoterpene	Eigenvector	Correlation coefficient (r)	*P*-value	Eigenvector	Correlation coefficient (r)	*P*-value
Bole	(-)-α-pinene	0.1119	0.3604	0.0224	0.1914	0.3524	0.0257
	(+)-α-pinene	0.4983	0.9008	< 0.0001*	0.0606	0.0626	0.7012
	(-)-camphene	-0.2326	-0.6291	< 0.0001*	0.2704	0.4180	0.0073
	(+)-camphene	0.0446	0.1001	0.5387	0.4742	0.6088	< 0.0001*
	(-)-sabinene	-0.2801	-0.5446	0.0003*	0.5008	0.5566	0.0002*
	(+)-sabinene	0.0023	0.0089	0.9567	0.1350	0.3	0.06
	(-)-β-pinene	0.4151	0.8880	< 0.0001*	0.1981	0.2422	0.1321
	(+)-β-pinene	0.2395	0.7213	< 0.0001*	0.2330	0.4013	0.0103
	myrcene	0.5655	0.9522	< 0.0001*	0.1174	0.1130	0.4875
	(+)- 3-carene	0.1217	0.2578	0.1083	-0.4655	-0.5638	0.0002*
	α-terpinene	-0.0137	-0.1052	0.5183	0.0013	0.0057	0.9720
	p-cymene	0.0034	0.0159	0.9226	-0.0388	-0.0829	0.6109
	(-)-β phellandrene	0.0623	0.2060	0.2023	0.0296	0.0561	0.7312
	(-)-limonene	0.1037	0.3716	0.0182	0.1155	0.2366	0.1417
	(+)-limonene	0.0161	0.0729	0.6546	0.1022	0.2645	0.0991
	γ-terpinene	0	0	0	0	0	0
	terpinolene	0.0118	0.1284	0.4298	-0.0127	-0.0792	0.6273

		Principal component 1			Principal component 2		
Tissue	Monoterpene	Eigenvector	Correlation coefficient (r)	*P*-value	Eigenvector	Correlation coefficient (r)	*P*-value
	(-)-bornyl acetate	0.1586	0.4086	0.0089	-0.2072	-0.3052	0.0556
	% total variance	41.46			13.55		
Foliage	(-)-α-pinene	0.1393	0.8229	< 0.0001*	0.0819	0.2762	0.0846
	(+)-α-pinene	0.1624	0.8684	< 0.0001*	0.0862	0.2628	0.1013
	(-)-camphene	0.4827	0.9340	< 0.0001*	0.2834	0.3128	0.0494
	(+)-camphene	0.3542	0.7834	< 0.0001*	-0.3167	-0.3996	0.0106
	(-)-sabinene	-0.3023	-0.6022	< 0.0001*	0.6421	0.7295	< 0.0001*
	(+)-sabinene	0.0376	0.1965	0.2243	0.1131	0.3372	0.0333
	(-)-β-pinene	-0.0017	-0.0074	0.9637	0.0582	0.1418	0.3829
	(+)-β-pinene	-0.0008	-0.0036	0.9825	0.0195	0.0487	0.7652
	myrcene	0.0789	0.5599	0.0002*	0.1190	0.4820	0.0016*
	(+)- 3-carene	-0.1259	-0.5340	0.0004*	0.2789	0.6746	< 0.0001*
	α-terpinene	-0.0527	-0.4595	0.0029*	-0.0486	-0.2415	0.1333
	p-cymene	-0.0478	-0.3935	0.0120	0.0317	0.1488	0.3595
	(-)-β phellandrene	0.0272	0.2292	0.1548	0.1002	0.4820	0.0016*
	(-)-limonene	0.2471	0.8362	< 0.0001*	0.1918	0.3703	0.0187
	(+)-limonene	0.2781	0.8974	< 0.0001*	0.1088	0.2002	0.2155
	γ-terpinene	-0.1058	-0.5368	0.0004*	0.2232	0.6456	< 0.0001*

		Principal component 1			Principal component 2		
Tissue	Monoterpene	Eigenvector	Correlation coefficient (r)	*P*-value	Eigenvector	Correlation coefficient (r)	*P*-value
	terpinolene	-0.1269	-0.4844	0.0015*	0.3185	0.6933	< 0.0001*
	(-)-bornyl acetate	0.5563	0.9417	< 0.0001*	0.2666	0.2575	0.1089
	% total variance	59.24			19.27		

Figure 4.5. Quantitative variation in bole monoterpenes of lodgepole pine, *Pinus contorta* var. *latifolia*, across three sites in British Columbia. Bars with different letters indicate significant differences in mean amounts of compounds across sites, REGW multiple comparisons test, $P < 0.05$, N = 10 per site. Bars with no letter indicate no difference across sites. Note variable scales on Y-axes.

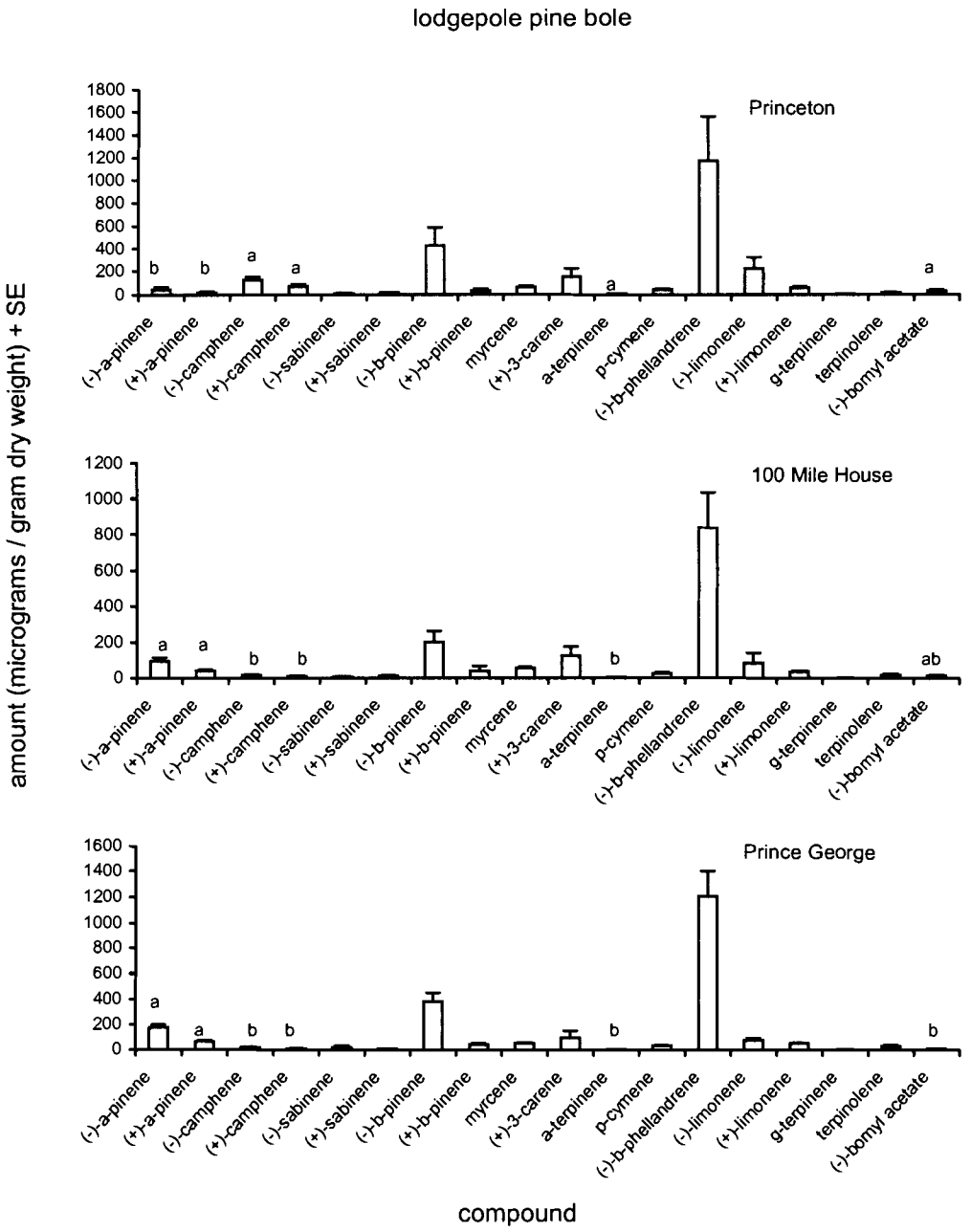
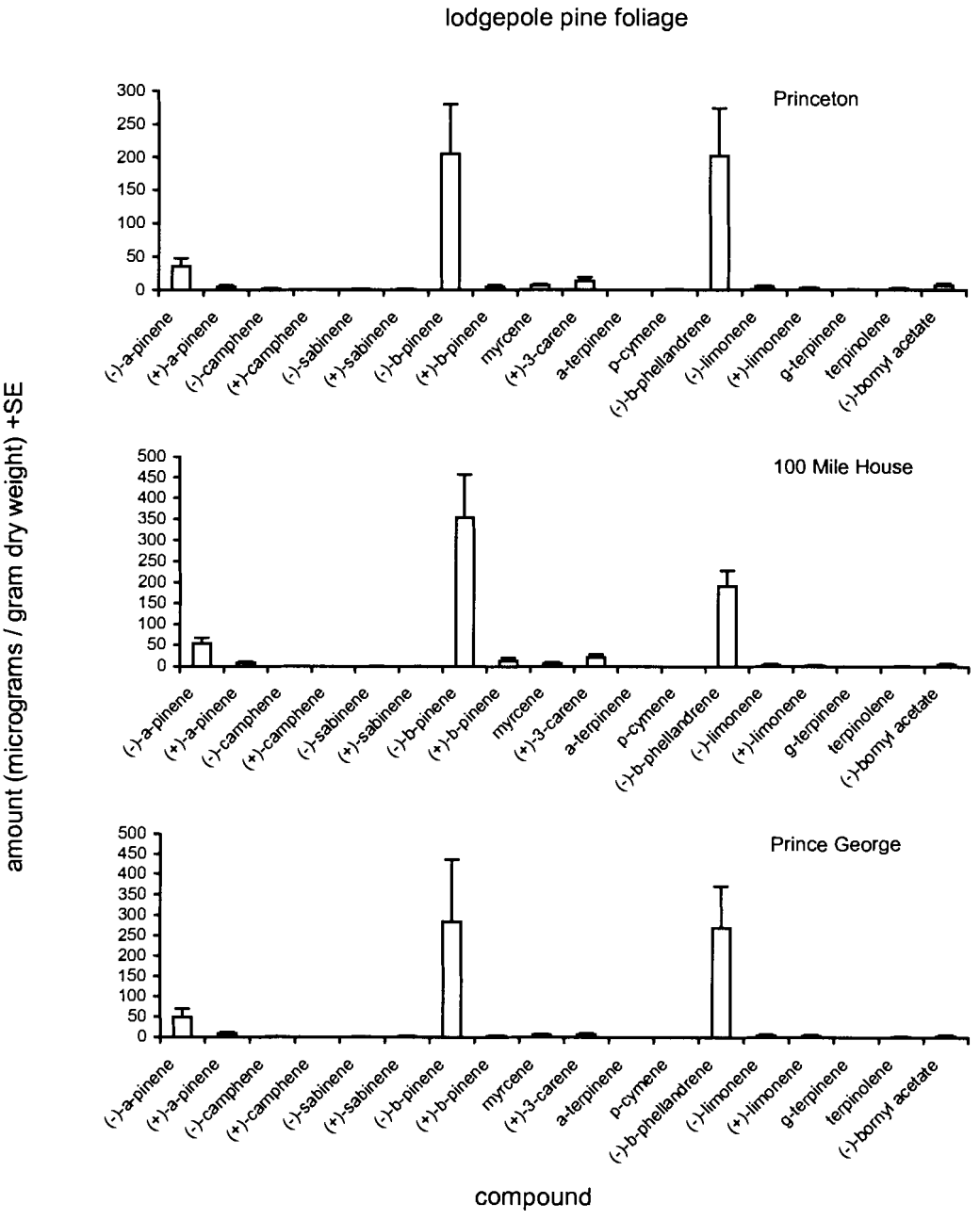

lodgepole pine bole
Princeton
100 Mile House
Prince George
amount (micrograms / gram dry weight) + SE
compound
(-)-a-pinene
(+)-a-pinene
(-)-camphene
(+)-camphene
(-)-sabinene
(+)-sabinene
(-)-b-pinene
(+)-b-pinene
myrcene
(+)-3-carene
a-terpinene
p-cymene
(-)-b-phellandrene
(-)-limonene
(+)-limonene
g-terpinene
terpinolene
(-)-bornyl acetate

Figure 4.6. Quantitative variation in foliage monoterpenes of lodgepole pine, *Pinus contorta* var. *latifolia*, across three sites in British Columbia. Bars with different letters indicate significant differences in mean amounts of compounds across sites, REGW multiple comparisons test, $P < 0.05$, N = 10 per site. Bars with no letter indicate no difference across sites. Note variable scales on Y-axes.


lodgepole pine foliage
Princeton
100 Mile House
Prince George
amount (micrograms / gram dry weight) +SE
compound
(-)-a-pinene
(+)-a-pinene
(-)-camphene
(+)-camphene
(-)-sabinene
(+)-sabinene
(-)-b-pinene
(+)-b-pinene
myrcene
(+)-3-carene
a-terpinene
p-cymene
(-)-b-phellandrene
(-)-limonene
(+)-limonene
g-terpinene
terpinolene
(-)-bornyl acetate

other components being α-pinene, β-pinene, and 3-carene. Forrest (1980) grouped North American lodgepole pines into 12 main types based on relative proportions of β-phellandrene, β-pinene and α-pinene in the oleoresin of bole tissues and terminal shoots of young trees grown from seed. My bole samples conform to his type B, where β-phellandrene > β-pinene > α-pinene; and my foliage samples to his type C, with β-pinene ≥ β-phellandrene > α-pinene. In his study, 3-carene and limonene were important components of oleoresin in the interior of B.C., but my bole and foliage samples contained < 6% and trace amounts, respectively, of both compounds, and high amounts of β-phellandrene.

In interior spruce from Prince George, there was significantly more (+)-α-pinene and (-)-limonene, and less (-)-β-phellandrene in bole volatiles than in trees from the other two sites (Figure 4.7.). In volatiles from foliage, Prince George trees had more (-)-bornyl acetate than trees from the other two sites, as well as more (+)-camphene and (+)-β-pinene, and less γ-terpinene than trees from 100 Mile House, and less (-)-β-phellandrene and terpinolene than trees from Princeton (Figure 4.8.).
γ-Terpinene and terpinolene were the only compounds that differed significantly between Princeton and 100 Mile House (Figure 4.8.). In bole volatiles, (+)-α-pinene predominated over its antipode in Prince George, while the reverse occurred in Princeton. Quantitative variation in monoterpenes among trees in different populations is due to genetic differences (Zavarin *et al.*, 1970; von Rudloff, 1972a; von Rudloff & Rehfeldt, 1980; Holm & Hiltunen, 1997; Chen *et al.*, 2002). I found no marked separation into

Figure 4.7. Quantitative variation in bole monoterpenes of interior spruce, *Picea engelmannii* x *glauca*, across three sites in British Columbia. Bars with different letters indicate significant differences in mean amounts of compounds across sites, REGW multiple comparisons test, $P < 0.05$, N = 10 per site. Bars with no letter indicate no difference across sites. Note variable scales on Y-axes.

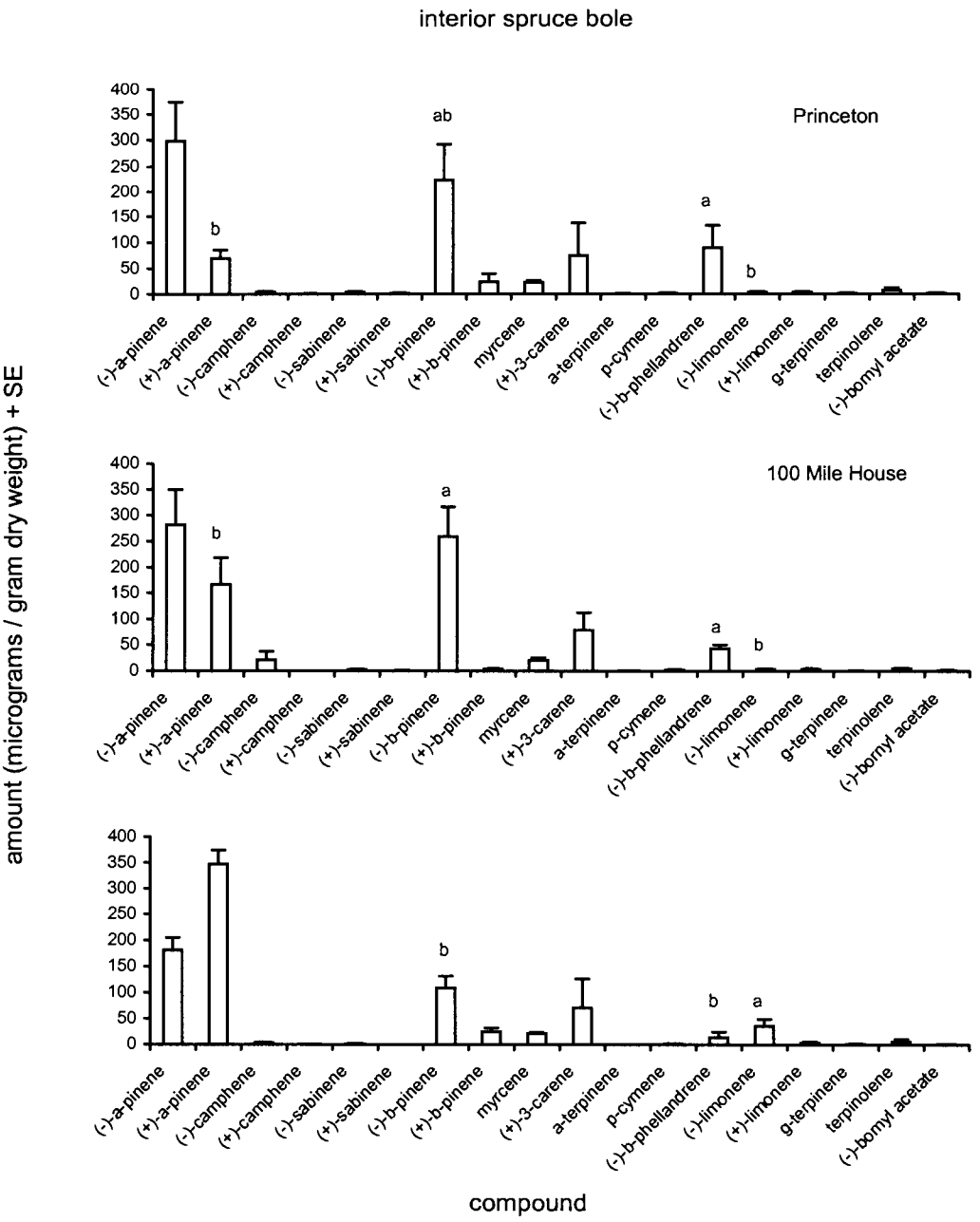
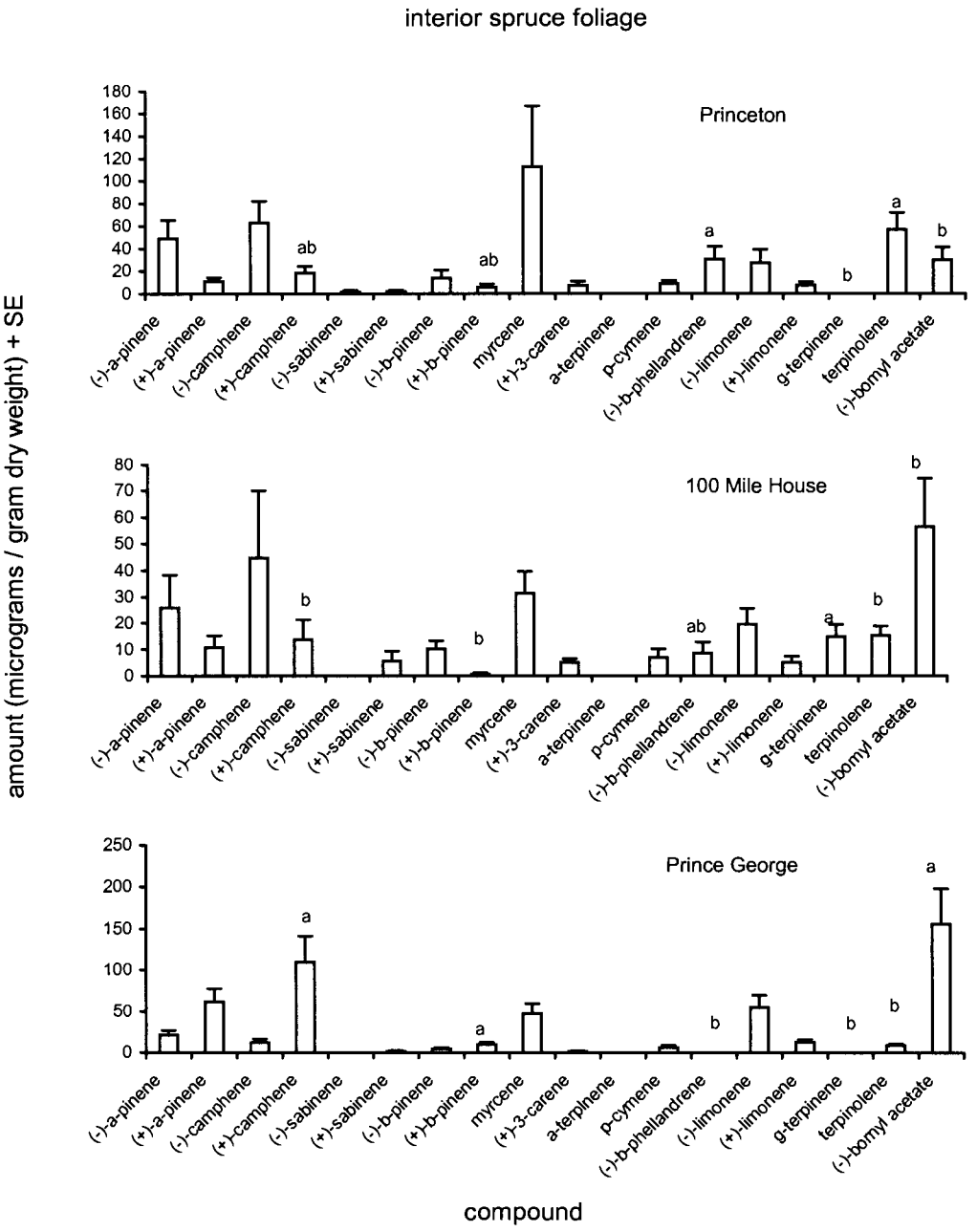

interior spruce bole
Princeton
100 Mile House
amount (micrograms / gram dry weight) + SE
compound
(-)-a-pinene
(+)-a-pinene
(-)-camphene
(+)-camphene
(-)-sabinene
(+)-sabinene
(-)-b-pinene
(+)-b-pinene
myrcene
(+)-3-carene
a-terpinene
p-cymene
(-)-b-phellandrene
(-)-limonene
(+)-limonene
g-terpinene
terpinolene
(-)-bornyl acetate

Figure 4.8. Quantitative variation in foliage monoterpenes of interior spruce, *Picea engelmannii* x *glauca*, across three sites in British Columbia. Bars with different letters indicate significant differences in mean amounts of compounds across sites, REGW multiple comparisons test, $P < 0.05$, N = 10 per site. Bars with no letter indicate no difference across sites. Note variable scales on Y-axes.


interior spruce foliage
Princeton
100 Mile House
Prince George
amount (micrograms / gram dry weight) + SE
compound
(-)-a-pinene
(+)-a-pinene
(-)-camphene
(+)-camphene
(-)-sabinene
(+)-sabinene
(-)-b-pinene
(+)-b-pinene
myrcene
(+)-3-carene
a-terpinene
p-cymene
(-)-b-phellandrene
(-)-limonene
(+)-limonene
g-terpinene
terpinolene
(-)-bornyl acetate

Engelmann and white spruce chemotypes, supporting Ogilvie & von Rudloff's (1968) chemosystematic study along the Bow River Valley, and Wright's (1955) observation of extensive introgression between Engelmann and white spruce in central and southern B.C.. Hybrid spruces, e.g. *P. glauca* x *P. engelmannii* (Ogilvie & von Rudloff, 1968), and *P. glauca* x *P. mariana* (Mill.) (Rosendahl spruce) (von Rudloff & Holst, 1968), have been documented to have intermediate quantitative monoterpene compositions compared to their parents.

Major monoterpenes in the bole of interior fir were (-)-β-pinene and (-)-β-phellandrene (Figure 4.9.). The percent composition of bole monoterpenes of interior fir was generally similar to the findings of Zavarin *et al.* (1970). Trees from Prince George had more (+)-camphene and (+)-β-pinene, and less limonene and terpinolene than trees in both Princeton and 100 Mile House, and less (-)-sabinene than trees from Princeton. There were no differences between trees in Princeton and 100 Mile House. Amounts of (-)-sabinene, myrcene and α-terpinene were significantly different between foliage of trees in Princeton and 100 Mile House (Figure 4.10.). Both enantiomers of camphene were significantly less abundant in trees in Prince George compared to those in Princeton, but there was no significant difference in any other compounds (Figure 4.10.). Trees in 100 Mile House and Prince George differed in the amounts of camphene, α-terpinene and (-)-bornyl acetate (Figure 4.10.).

Principal components analyses of the entire complement of bole and foliage volatile profiles did not reveal significant separation among sites for lodgepole pine, interior spruce or interior fir.

Figure 4.9. Quantitative variation in bole monoterpenes of interior fir, *Abies lasiocarpa* x *bifolia*, across three sites in British Columbia. Bars with different letters indicate significant differences in mean amounts of compounds across sites, REGW multiple comparisons test, $P < 0.05$, N = 10 per site. Bars with no letter indicate no difference across sites. Note variable scales on Y-axes.

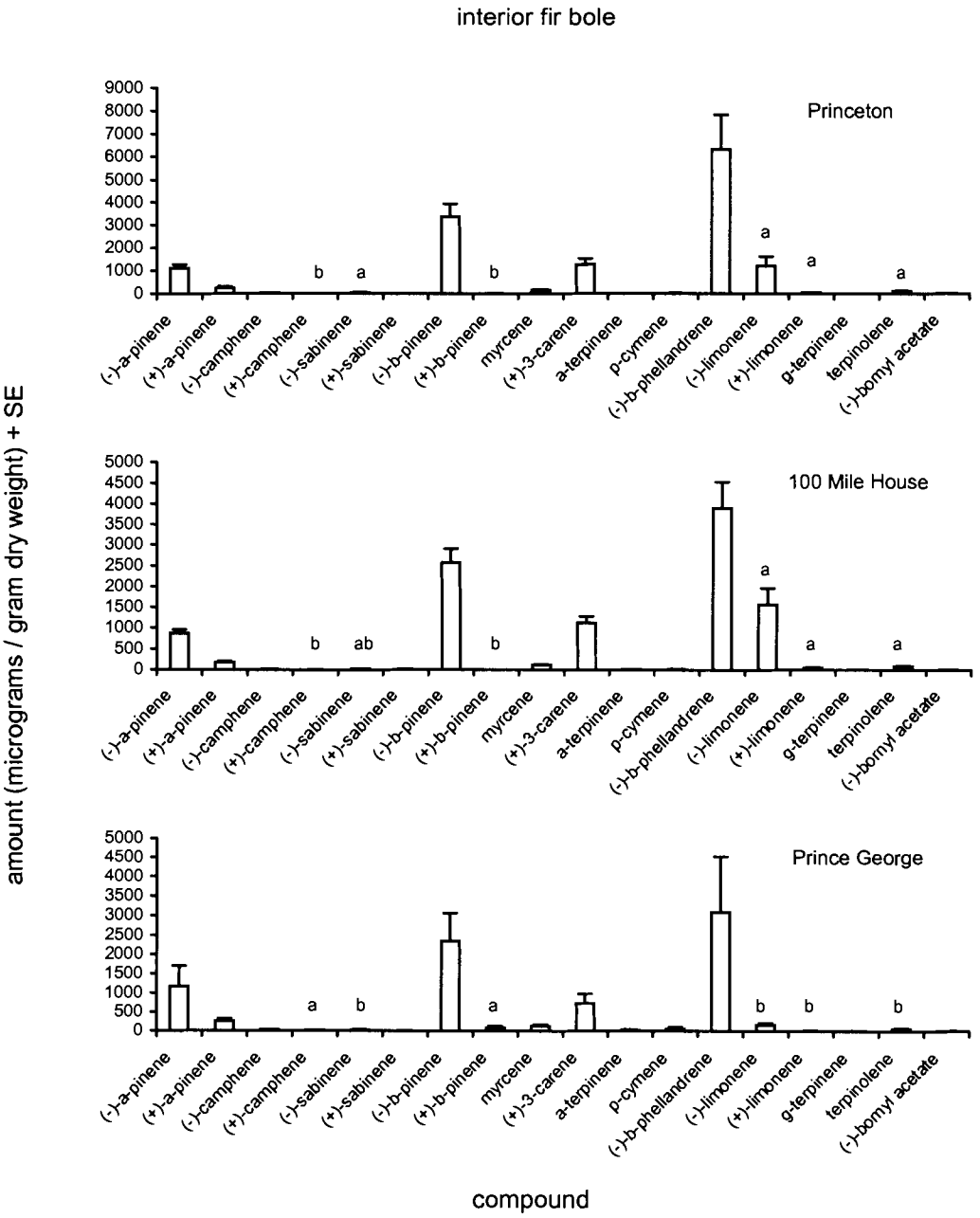
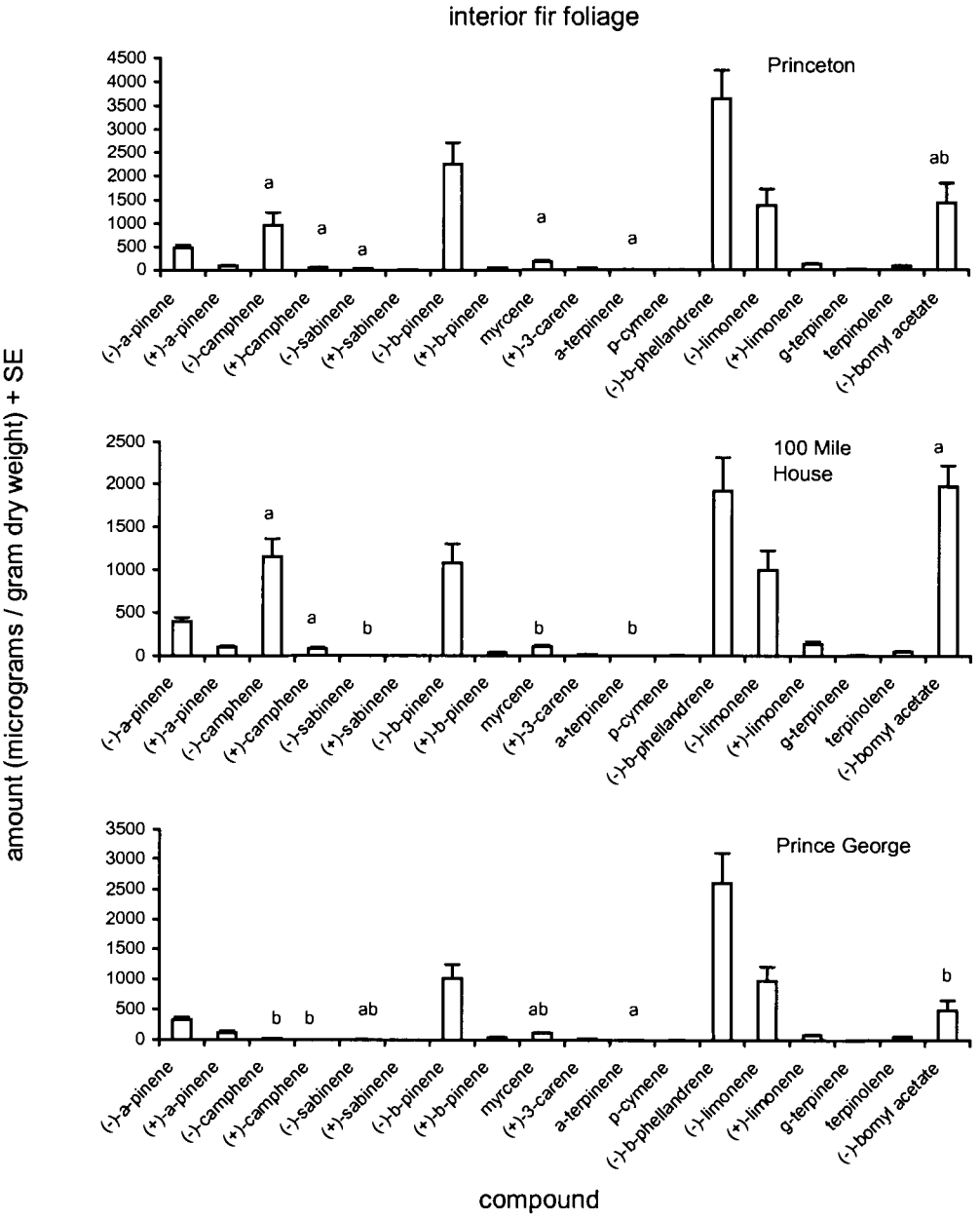

interior fir bole
Princeton
100 Mile House
Prince George
amount (micrograms / gram dry weight) + SE
compound
(-)-a-pinene
(+)-a-pinene
(-)-camphene
(+)-camphene
(-)-sabinene
(+)-sabinene
(-)-b-pinene
(+)-b-pinene
myrcene
(+)-3-carene
a-terpinene
p-cymene
(-)-b-phellandrene
(-)-limonene
(+)-limonene
g-terpinene
terpinolene
(-)-bornyl acetate

Figure 4.10. Quantitative variation in foliage monoterpenes of interior fir, *Abies lasiocarpa* x *bifolia*, across three sites in British Columbia. Bars with different letters indicate significant differences in mean amounts of compounds across sites, REGW multiple comparisons test, $P < 0.05$, N = 10 per site. Bars with no letter indicate no difference across sites. Note variable scales on Y-axes.


interior fir foliage
Princeton
100 Mile House
Prince George
amount (micrograms / gram dry weight) + SE
compound
(-)-a-pinene
(+)-a-pinene
(-)-camphene
(+)-camphene
(-)-sabinene
(+)-sabinene
(-)-b-pinene
(+)-b-pinene
myrcene
(+)-3-carene
a-terpinene
p-cymene
(-)-b-phellandrene
(-)-limonene
(+)-limonene
g-terpinene
terpinolene
(-)-bornyl acetate

Variation among species

Multivariate analyses of variance indicated that the bole and folilage volatile profiles were differed significantly among species (bole: Wilks' $\lambda = 0.0045$, $F = 25.82$, $df = 54$, 271.96, $P < 0.0001$; foliage: Wilks' $\lambda = 0.00079$, $F = 50.51$, $df = 54$, 271.96, $P < 0.0001$). Amounts of (+)-camphene, (-)-β-pinene, myrcene, (+)-3-carene, (-)-β-phellandrene and terpinolene in bole volatiles were significantly different among all four species (Table 4.4.). The other 12 compounds in the bole were significantly different between at least two of the four species. In foliage, (+)-Sabinene was the only compound that did not differ in amount among species (Table 4.4.). (+)-α-Pinene, (-)-camphene, (-)-β-phellandrene, both enantiomers of limonene and γ-terpinene were significantly different in all four species. The remaining 11 compounds differed significantly in at least two of the four species.

Quantitative variation in relative monoterpene amounts occurs among species of conifers. Accordingly, differences in relative amounts of monoterpenes among species were found in nine *Larix* spp. although they did not differ qualitatively (Holm & Hiltunene, 1997). Eleven monoterpenes in sap and heartwood were common to both *P. contorta* and knobcone pine, *Pinus attenuata* Lemmon, but they differed in relative amounts of their major constituents, 71% β-phellandrene in *P. contorta* and 72% α-pinene in *P. attenuata* (Anderson *et al.*, 1969). Four other species, *P. sylvestris, Pinus yunanensis* Franchet, *Pinus armandi* Franchet and Australes, and *Pinus caribaea* var. *caribaea* Morelet differed from each other based on relative terpene amounts (Faldt *et al.*,

Table 4.4. Results of statistical analyses of variation in the amount of monoterpenes in the bole and foliage of four species of conifers in interior B.C. df = 3, 108, F and *P* values from multivariate analyses of variance. For each monoterpene, species with the same letter are not significantly different, REGW test, $P < 0.05$.

Tissue	Monoterpene	Species	Multiple comparisons A (high) to D (low)	F	*P*
Bole	(-)-α-pinene	Douglas-fir	C	65.94	< 0.0001
		lodgepole pine	C		
		interior spruce	B		
		interior fir	A		
	(+)-α-pinene	Douglas-fir	B	29.97	< 0.0001
		lodgepole pine	C		
		interior spruce	A		
		interior fir	A		
	(-)-camphene	Douglas-fir	B	19.84	< 0.0001
		lodgepole pine	A		
		interior spruce	C		
		interior fir	B		
	(+)-camphene	Douglas-fir	B	34.54	< 0.0001
		lodgepole pine	A		
		interior spruce	D		
		interior fir	C		

Tissue	Monoterpene	Species	Multiple comparisons A (high) to D (low)	F	*P*
	(-)-sabinene	Douglas-fir	C	14.14	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		
	(+)-sabinene	Douglas-fir	C	25.91	< 0.0001
		lodgepole pine	A		
		interior spruce	C		
		interior fir	B		
	(-)-β-pinene	Douglas-fir	D	153.65	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		
	(+)-β-pinene	Douglas-fir	B	4.14	0.0080
		lodgepole pine	A		
		interior spruce	AB		
		interior fir	B		
	myrcene	Douglas-fir	D	118.89	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		

Tissue	Monoterpene	Species	Multiple comparisons A (high) to D (low)	F	*P*
	(+)-limonene	Douglas-fir	C	55.76	< 0.0001
		lodgepole pine	A		
		interior spruce	C		
		interior fir	B		
	γ-terpinene	Douglas-fir	C	60.07	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		
	terpinolene	Douglas-fir	D	118.15	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		
	(-)-bornyl acetate	Douglas-fir	B	26.11	< 0.0001
		lodgepole pine	A		
		interior spruce	B		
		interior fir	A		
Foliage	(-)-α-pinene	Douglas-fir	A	78.85	< 0.0001
		lodgepole pine	B		
		interior spruce	B		
		interior fir	A		

Tissue	Monoterpene	Species	Multiple comparisons A (high) to D (low)	F	*P*
	(+)-α-pinene	Douglas-fir	B	44.83	< 0.0001
		lodgepole pine	D		
		interior spruce	C		
		interior fir	A		
	(-)-camphene	Douglas-fir	A	181.13	< 0.0001
		lodgepole pine	D		
		interior spruce	C		
		interior fir	B		
	(+)-camphene	Douglas-fir	A	23.67	< 0.0001
		lodgepole pine	B		
		interior spruce	A		
		interior fir	A		
	(-)-sabinene	Douglas-fir	A	47.73	< 0.0001
		lodgepole pine	C		
		interior spruce	C		
		interior fir	B		
	(+)-sabinene	Douglas-fir	A	2.66	0.0521
		lodgepole pine	A		
		interior spruce	A		
		interior fir	A		

Tissue	Monoterpene	Species	Multiple comparisons A (high) to D (low)	F	*P*
	(-)-β-pinene	Douglas-fir	A	66.34	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		
	(+)-β-pinene	Douglas-fir	B	24.93	< 0.0001
		lodgepole pine	C		
		interior spruce	C		
		interior fir	A		
	myrcene	Douglas-fir	B	51.62	< 0.0001
		lodgepole pine	C		
		interior spruce	B		
		interior fir	A		
	(+)-3-carene	Douglas-fir	BC	14.38	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		
	α-terpinene	Douglas-fir	B	25.32	< 0.0001
		lodgepole pine	B		
		interior spruce	B		
		interior fir	A		

Tissue	Monoterpene	Species	Multiple comparisons A (high) to D (low)	F	*P*
	(+)-3-carene	Douglas-fir	D	82.24	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		
	α-terpinene	Douglas-fir	C	55.26	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		
	p-cymene	Douglas-fir	C	103.07	< 0.0001
		lodgepole pine	A		
		interior spruce	C		
		interior fir	B		
	(-)-β-phellandrene	Douglas-fir	D	236.50	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		
	(-)-limonene	Douglas-fir	C	103.20	< 0.0001
		lodgepole pine	B		
		interior spruce	C		
		interior fir	A		

Tissue	Monoterpene	Species	Multiple comparisons A (high) to D (low)	F	*P*
	p-cymene	Douglas-fir	B	28.65	< 0.0001
		lodgepole pine	B		
		interior spruce	A		
		interior fir	A		
	(-)-β-phellandrene	Douglas-fir	C	140.94	< 0.0001
		lodgepole pine	B		
		interior spruce	D		
		interior fir	A		
	(-)-limonene	Douglas-fir	B	135.55	< 0.0001
		lodgepole pine	D		
		interior spruce	C		
		interior fir	A		
	(+)-limonene	Douglas-fir	B	105.17	< 0.0001
		lodgepole pine	D		
		interior spruce	C		
		interior fir	A		
	γ-terpinene	Douglas-fir	B	54.87	< 0.0001
		lodgepole pine	D		
		interior spruce	C		
		interior fir	A		

Tissue	Monoterpene	Species	Multiple comparisons A (high) to D (low)	F	*P*
	terpinolene	Douglas-fir	A	118.56	< 0.0001
		lodgepole pine	C		
		interior spruce	B		
		interior fir	A		
	(-)-bornyl acetate	Douglas-fir	A	143.74	< 0.0001
		lodgepole pine	C		
		interior spruce	B		
		interior fir	A		

2001). Except for some overlap between Douglas-fir and interior spruce, particularly for bole volatiles, principal components analysis of the entire monoterpene complement of the four species in my study separated species based on both bole and foliage volatile profiles (Figure 4.11.).

Correlation analyses of monoterpene amounts with the first two principal components (Table 4.5.) revealed that for bole volatiles, the PC 1 distinguished between species with high relative amounts of all monoterpenes (except (+)-α-pinene, (+)-camphene, and (-)-β-pinene), i.e. lodgepole pine and interior fir, compared to Douglas-fir and interior spruce. PC 2 separated species containing low amounts of both enantiomers of α-pinene, i.e. lodgepole pine, from the other three speices. For foliage volatiles, there was high positive correlation of all monoterpenes with PC 1. PC 1 distinguished between species with trees containing high overall amounts of all monoterpenes, i.e., Douglas-fir and interior fir, from lodgepole pine and interior spruce (Figure 4.11.). PC 2 separates out species with low amounts of both enantiomers of camphene, i.e. lodgepole pine and interior fir from interior spruce and Douglas-fir.

Multivariate analyses e.g. principal components analysis could be used by investigators to bring statistical precision to chemosystematic analyses. My results also demonstrate the risk of placing too much faith on chemosystematics based on limited sampling. Thus, only the results of my foliage profiles support Hart's (1987) cladistic relationship of Pinaceae (based on classical systematics), in which *Pinus* and *Picea* share a more recent common ancestor than the clade giving rise to *Pseudotsuga* and *Abies*.

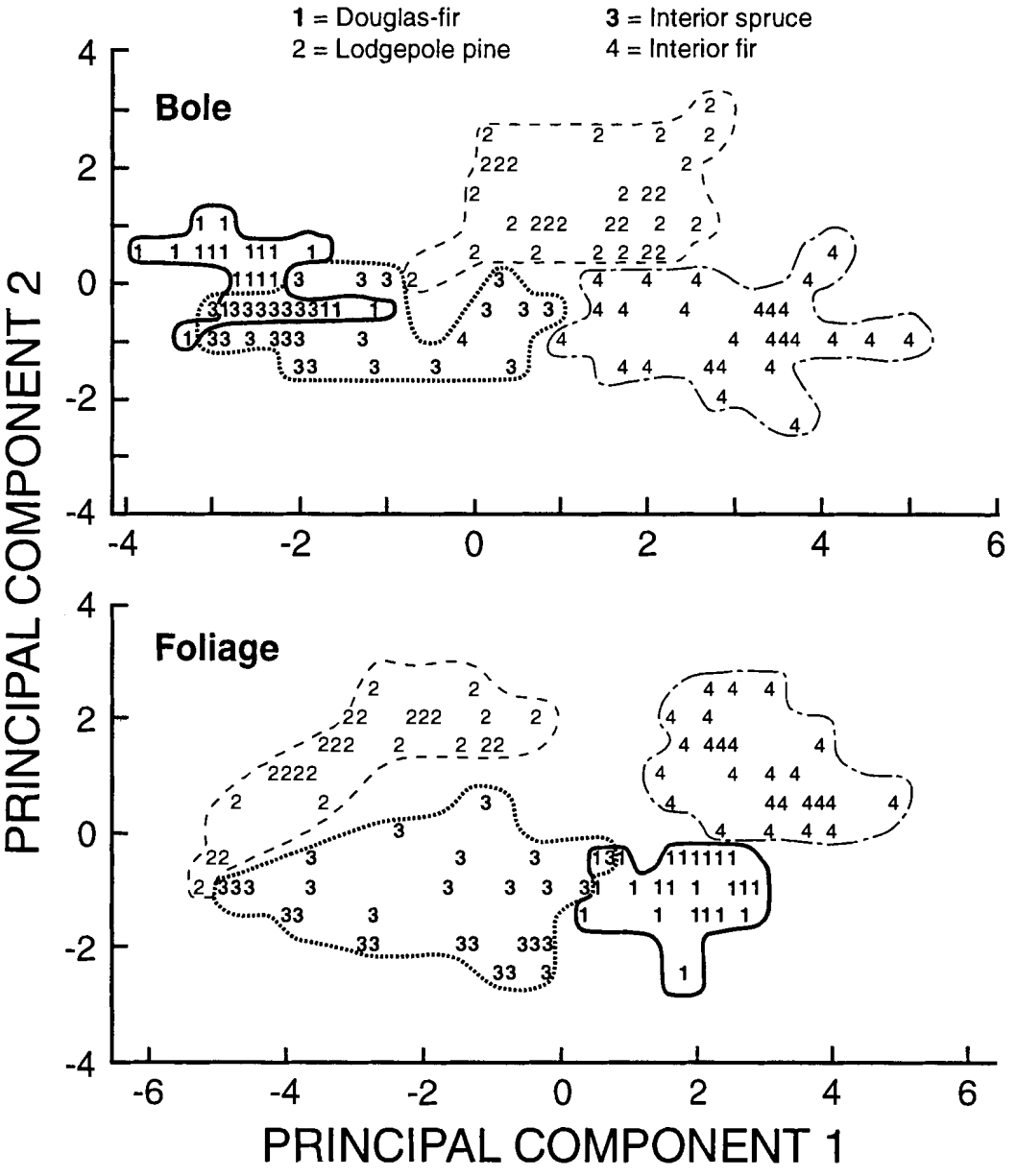
Table 4.5. Eigenvectors, correlation coefficients of monoterpenes in bole and foliage of four species of conifers with the first two principal components. Sequential Bonferroni adjusted $\alpha \leq 0.05$. Asterisks indicate significance.

		Principal component 1			Principal component 2		
Tissue	Monoterpene	Eigenvector	Correlation coefficient (r)	*P*-value	Eigenvector	Correlation coefficient (r)	*P*-value
Bole	(-)-α-pinene	0.1080	0.4337	< 0.0001*	-0.3499	-0.6296	< 0.0001*
	(+)-α-pinene	0.0155	0.0650	0.4806	-0.3504	-0.6598	< 0.0001*
	(-)-camphene	0.1003	0.3942	< 0.0001*	0.3600	0.6339	< 0.0001*
	(+)-camphene	0.0290	0.1106	0.2290	0.4393	0.7495	< 0.0001*
	(-)-sabinene	0.1702	0.6202	< 0.0001*	-0.0139	-0.0227	0.8059
	(+)-sabinene	0.1273	0.5762	< 0.0001*	0.2249	0.4559	< 0.0001*
	(-)-β-pinene	0.2517	0.8272	< 0.0001*	-0.2035	-0.2994	0.0009*
	(+)-β-pinene	-0.0219	-0.0705	0.4444	0.2233	0.3215	0.0003*
	myrcene	0.1963	0.8122	< 0.0001*	-0.07856	-0.1456	0.1126
	(+)- 3-carene	0.4326	0.8929	< 0.0001*	-0.2318	-0.2143	0.0188
	α-terpinene	0.1894	0.8523	< 0.0001*	0.0224	0.0452	0.6244
	p-cymene	0.2179	0.7498	< 0.0001*	0.2891	0.4456	< 0.0001*
	(-)-β phellandrene	0.5064	0.9450	< 0.0001*	0.0915	0.0765	0.4064
	(-)-limonene	0.3307	0.8384	< 0.0001*	-0.0740	-0.0840	0.3617
	(+)-limonene	0.2146	0.7222	< 0.0001*	0.2727	0.4111	< 0.0001*
	γ-terpinene	0.1721	0.8806	< 0.0001*	-0.0525	-0.1203	0.1906
	terpinolene	0.3083	0.9418	< 0.0001*	-0.0996	-0.1362	0.1380
	(-)-bornyl acetate	0.1504	0.6244	< 0.0001*	0.2128	0.3957	< 0.0001*

Tissue	Monoterpene	Principal component 1			Principal component 2		
		Eigenvector	Correlation coefficient (r)	*P*-value	Eigenvector	Correlation coefficient (r)	*P*-value
	% total variance	57.90			11.62		
Foliage	(-)-α-pinene	0.2855	0.9123	< 0.0001*	0.0811	0.1318	0.1515
	(+)-α-pinene	0.2259	0.8691	< 0.0001*	-0.0242	-0.0474	0.6070
	(-)-camphene	0.4027	0.8777	< 0.0001*	-0.3115	-0.3451	0.0001*
	(+)-camphene	0.1718	0.5381	< 0.0001*	-0.3510	-0.5589	< 0.0001*
	(-)-sabinene	0.1656	0.4798	< 0.0001*	-0.0637	-0.0939	0.3079
	(+)-sabinene	0.0448	0.2946	0.0011*	-0.0346	-0.1156	0.2086
	(-)-β-pinene	0.2938	0.7296	< 0.0001*	0.4109	0.5188	< 0.0001*
	(+)-β-pinene	0.1808	0.7001	< 0.0001*	0.1144	0.2252	0.0134
	myrcene	0.2016	0.8177	< 0.0001*	-0.0164	-0.0338	0.7144
	(+)- 3-carene	0.0820	0.3971	< 0.0001*	0.2125	0.5232	< 0.0001*
	α-terpinene	0.0316	0.3053	0.0007*	0.0767	0.3763	< 0.0001*
	p-cymene	0.0376	0.2325	0.0106*	-0.0512	-0.1608	0.0793
	(-)-β phellandrene	0.2402	0.5511	< 0.0001*	0.6658	0.7768	< 0.0001*
	(-)-limonene	0.3608	0.9097	< 0.0001*	-0.0018	-0.0023	0.9801
	(+)-limonene	0.2767	0.9320	< 0.0001*	-0.0161	-0.0276	0.7646
	γ-terpinene	0.1142	0.6173	< 0.0001*	0.0160	0.0439	0.6337
	terpinolene	0.2074	0.7667	< 0.0001*	-0.1400	-0.2632	0.0037*

		Principal component 1			Principal component 2		
Tissue	Monoterpene	Eigenvector	Correlation coefficient (r)	*P*-value	Eigenvector	Correlation coefficient (r)	*P*-value
	(-)-bornyl acetate	0.3964	0.9062	< 0.0001*	-0.2609	-0.3032	0.0008*
	% total variance	58.02			15.00		

Figure 4.11. Principal components analysis of entire complement of bole and foliage monoterpenes of Douglas-fir, *Pseudotsuga menziesii*, lodgepole pine, *Pinus contorta* var. *latifolia*, interior spruce *Picea engelmannii* x *glauca*, and interior fir, *Abies lasiocarpa* x *bifolia.* PC1 and PC2 account for 57.9 % and 11.6 %, respectively, of variation in bole volatiles and 58 % and 15 %, respectively, of variation in foliage volatiles. Polygons are drawn around identical numbers to aid in showing separation or overlap among species.


1 = Douglas-fir
2 = Lodgepole pine
3 = Interior spruce
4 = Interior fir
Bole
Foliage
PRINCIPAL COMPONENT 2
PRINCIPAL COMPONENT 1

4.4. Monoterpenes and Herbivory

Enantiomeric composition of host monoterpenes like α-pinene, may determine the production of oxidised metabolites as bark beetle pheromones. (-)-α-Pinene in *I. paraconfusus* and *I. typographus* is metabolised to the aggregation pheromone *cis*-verbenol, while (+)-α-pinene is converted into *trans*-verbenol (Renwick *et al.*, 1976a; Klimetzek & Francke, 1980). Many *Dendroctonus* spp. however, can convert either enantiomer into *trans*-verbenol (Hughes, 1973; Byers, 1983; Gries *et al.*, 1990). Predominance of (-)-α-pinene in conifer tissues I sampled, except for equal amounts of both enantiomers in Douglas-fir bole tissues in 100 Mile House and Prince George, and prevalence of (+)-α-pinene in both bole and foliage of spruce in Prince George, may influence the species of bark beetles that can exploit them as hosts.

Monoterpene composition in conifers does not vary with time, remains unchanged from late summer till the end of winter, and is not influenced by edaphic factors, making it ideal for sample collection and analysis from a chemosystematic perspective (von Rudloff, 1975; Smith, 2000). My results suggest that detection of quantitative differences in monoterpene profiles could also be used by specialist herbivores to distinguish between hosts and nonhosts, as well as resistant and susceptible hosts (Tomlin *et al.*, 1997), if constitutive monoterpenes reflect emitted profiles. Baier *et al.* (1999) concluded that internal composition of monoterpenes in Norway spruce, *P. abies*, was not reflected in the composition of emitted monoterpenes. However, when I performed a rank correlation analysis on their published data, a correlation coefficient (r) of 0.75, $P =$

0.0085, between mean amounts of constitutive and emitted terpenes indicated that emitted profiles do indeed reflect internal compostion.

Quantitative composition of monoterpenes can determine whether herbivores can successfully overcome their hosts. Among eight populations of *P. ponderosa* in California and Oregon, trees in areas with a history of infestation by the western pine beetle, *D. brevicomis* contained high levels of toxic limonene, and low levels of α-pinene in their xylem oleoresin (Sturgeon, 1979). Similarly, maritime pines, *P. pinaster* attacked by the European stem borer, *Dioryctria sylvestrella* Ratz. (Lepidoptera: Pyralidae), had significantly more limonene, longipinene, and copaene, and less camphene than resistant trees (Jactel *et al.*, 1996). Among resistant clones of Douglas-fir, potential fitness of the western spruce budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae), was lower on trees with high total monoterpenes (Chen *et al.*, 2002). However, Sitka spruce, *Picea sitchensis* Bong (Carr), in resistant provenances, had lower feeding rates (Tomlin & Borden, 1996) and lower monoterpene content than in susceptible provenances, suggesting that in this case, resistance to feeding by the white pine weevil, *Pissodes strobi* (Peck) (Coleoptera: Curculionidae), could be imparted by a relative lack of chemical apparency to the herbivore (Tomlin *et al.*, 1997). Japanese scale insects *Fiorinia externa* Ferris and *Nuculaspis tsugae* (Marlatt) (Homoptera: Diaspididae) had higher fecundity on their Japanese host, *Tsuga sieboldii* Carrière, which was relatively rich in terpene alcohols compared to the North American host, *Tsuga canadensis* (L.) Carr, which was rich in terpene hydrocarbons and acetates (McClure & Hare, 1984), indicating that coevolutionary relationships between trees and their

herbivores, could result in directional selection for trees with unfavourable monoterpene compositions in herbivore infested areas.

My study reveals significant quantitative differences in relative monoterpene amounts between coastal and interior Douglas-fir, as well as among interior Douglas-fir, lodgepole pine, interior spruce and interior fir that occur in sympatry over wide geographic areas. Differences are sufficiently great to justify investigation of the hypothesis (tested in Chapter 5), that bark beetles could potentially use these differences as discriminatory cues in host selection.

5. Primary attraction and kairomonal host discrimination

5.1. Introduction

Bark beetles must locate suitable trees of their host species before they succumb to dehydration, exhaustion or predation during dispersal. Whether bark beetles select such hosts by primary attraction to host volatiles, or by landing on them at random and sampling them for suitability has been an enduring question among bark beetle researchers (Person, 1931; Chapman, 1963; D.L. Wood, 1976, 1982; Gries *et al.*, 1989; Byers, 1996; Brattli *et al.*, 1998). Beetles have been documented to land randomly on host and nonhost conifers, and leave if they are unsuitable (Bunt *et al.*, 1980; Elkinton & Wood, 1980; Hynum & Berryman, 1980). Some are also attracted to ethanol and monoterpenes from host conifers (Rudinsky, 1966c; Byers *et al.*, 1985; Chénier & Philogène, 1989).

Beetles may be deterred from responding to their aggregation pheromones in the presence of volatiles from unsuitable hosts or nonhosts (Klimetzek *et al.*, 1986; Schroeder & Lindelow, 1989; Byers, 1995; Byers *et al.*, 1998). Bark and foliage volatiles from nonhost birches, *B. pendula* and *Betula pubescens* Ehrh., inhibited the attraction of *Pityogenes chalcographus* (L.) and *I. typographus* to their aggregation pheromones (Byers *et al.*, 1998). Green leaf volatiles inhibited the response of *D. frontalis*, *Ips grandicollis* (Eichhoff), *Ips avulsus* (Eichhoff) (Dickens *et al.*, 1991, 1992), *I. pini* (Huber *et al.*, 2001), *D. ponderosae* (Wilson *et al.*, 1996; Borden *et al.*, 1998; Huber &

Borden, 2001b), *D. pseudotsugae* (Huber & Borden, 2001a), *D. rufipennis*, *D. brevicomis* (Poland *et al.*, 1998), *Trypodendron lineatum* (Olivier) (Borden *et al.*, 1997), *P. bidentatus* (Byers *et al.*, 2000), *I. typographus* (Schlyter *et al.*, 1995; Zhang *et al.*, 1999; Zhang & Schlyter, 2003), and *Ips duplicatus* Sahlb.(Zhang *et al.*, 2001) to their aggregation pheromones, and *Tomicus piniperda* (L.) to host monoterpenes (Schlyter *et al.*, 1995). Odours from nonhost aspen, *Populus tremula* L, and birch, *B. pendula,* inhibited the response of *T. piniperda* to ethanol and cut bolts of Scots pine, *P. sylvestris* (Schroeder & Lindelow, 1989; Byers, 1992; Schroeder, 1992).

Pioneer beetles at the beginning of flight cannot rely solely on aggregation pheromones produced by their counterparts to locate hosts. Moreover, several species of bark beetles have overlapping flight periods, and share common pheromone components. For example, both Douglas-fir beetles, *D. pseudotsugae,* and spruce beetles, *D. rufipennis*, respond to frontalin (Pitman & Vité, 1970; Dyer, 1973, 1975) and MCOL (1-methylcyclohex-2-en-1-ol) (Libbey *et al.*, 1983; Setter & Borden, 1999). Thus, if host discrimination occurs at long range, pioneer beetles and followers would have to discriminate among sympatric host and nonhost conifers by their volatile constituents, e.g. monoterpene profiles. Primary attraction to host monoterpenes and the ability to discriminate among sympatric conifers would also reduce searching costs, and allow beetles to quickly embark on reproduction.

Detailed studies are required to decipher the intricacies of the host selection process (Byers *et al.*, 1998, 2000). While recent studies (cited above) have investigated repellent properties of nonhost angiosperm volatiles on the aggregation behaviour of

coniferophagous bark beetles, and some studies have focussed on how beetles identify susceptible trees of the host species (Hynum & Berryman, 1980; Moeck *et al.*, 1981; Gara *et al.*, 1984; Miller *et al.*, 1986), relatively few studies (Chapman, 1963; Elkinton & Wood, 1980) have explored the basis by which these beetles discriminate among sympatric species of host and nonhost conifers.

In Chapter 3, an examination of the volatile chemical profiles of the above conifers and the antennal responses of the three species of beetles to these compounds revealed no significant qualitative differences either in the chemistry of the four species of conifers or in the beetles' antennal responses to these compounds. However, in Chapter 4, I found that the conifer species differed so significantly in their quantitative monoterpene profiles, that in this Chapter, I hypothesised that bark beetles could potentially use this information to discriminate among them. This raised the question as to whether bark beetles can actually respond to these differences in flight.

Following up results from investigations in Chapter 4, I conducted 18 field-trapping experiments (reported in this Chapter) with three species of *Dendroctonus* that tested whether: 1) primary attraction occurred in response to bole and foliage volatiles of host conifers, and 2) if given a choice of an attractive pheromone bait in combination with volatiles from host and nonhost conifers, whether beetles could discriminate among them, and orient toward the right host species. Results for *Dr. confusus* are reported in the Appendix, as beetles were not captured in sufficient numbers to draw definite conclusions.

5.2. Materials and methods

Preparation of synthetic blends

Fourteen monoterpenes (Table 5.1.), which each constituted ≥ 5% of the volatiles of bole or foliage volatiles of the four species of conifers (Chapter 4), were mixed together in their estimated natural proportions (Table 5.2.). Almost all chemicals were commercially available except for (-)-sabinene, which constitutes > 30% of the foliage of coastal Douglas-fir, and (-)-β-phellandrene, which constitutes ~ 60% of lodgepole pine bole volatiles. These two compounds were distilled from natural sources by H.D. Pierce, Jr., Department of Biological Sciences, Simon Fraser University.

To obtain (-)-sabinene, Douglas-fir needle oil (Liberty Natural Products, Portland, OR 97215) was fractionated through a 30 cm Dufton column fitted with a total reflux, partial take-off head at 100 mm Hg. Fractions were taken off periodically and analysed by gas chromatography for (-)-sabinene content. The distillate (bp. 90-100°C) was a mixture primarily of α-pinene, β-pinene, and (-)-sabinene, along with small amounts of other common terpenes. Black pepper oil (Liberty Natural Products, Portland, OR 97215) was also used to isolate fractions enriched in (-)-sabinene, but was less desirable due to higher levels of 3-carene and limonene, compared to Douglas-fir needle oil. (-)-Sabinine constituted 19-23% of both distillates.

To obtain (-)-β-phellandrene, raw turpentine, primarily from lodgepole pine pulp (Prince George Pulp and Paper Mills, Prince George, B.C. V2N 2K3), containing 41% (-)-β-phellandrene, was distilled at ca. 30 mm Hg in a fume hood. Aliquots of the distillate, 1.5 L each, were transferred to a 2 L separatory funnel and washed extensively

Table 5.1. Monoterpenes in conifer blends, purity and source.

Monoterpene	Purity (%)	Source
(-)-α-pinene	98	Aldrich Chemical Co.
(+)-α-pinene	98	Aldrich Chemical Co.
(-)-camphene	80	Aldrich Chemical Co.
(+)-camphene	80	Aldrich Chemical Co.
(-)-sabinene	19-23	H.D. Pierce, Jr.
(-)-β-pinene	99	Aldrich Chemical Co.
myrcene	90	Sigma Chemical Co.
(+)-3-carene	90	Aldrich Chemical Co.
p-cymene	99	Aldrich Chemical Co.
(-)-β-phellandrene	60-65	H.D. Pierce, Jr.
(-)-limonene	96	Aldrich Chemical Co.
γ-terpinene	95	Aldrich Chemical Co.
terpinolene	90	Fluka Chemical Co.
(-)-bornyl acetate	97	Sigma Chemical Co.

Table 5.2. Actual and realised composition of conifer blends.

	Bole volatiles			Foliage volatiles	
Compound[a]	Actual composition (%)	Realised composition (%)	Compound	Actual composition (%)	Realised composition (%)
coastal Douglas-fir (CDF)					
(-)-α-pinene	72.7	68.62	(-)-α-pinene	11.49	7.78
(-)-camphene	13.62	12.94	(-)-sabinene	33.51	22.12
(-)-sabinene	5.87	5.13	(-)-β-pinene	42.64	45.98
(-)-β-pinene	7.82	9.22	terpinolene	12.35	7.94
		others: 4.09			(+)-3-carene: 11.95
					myrcene: 2.3
interior Douglas-fir (IDF)					
(-)-α-pinene	37.52	37.5	(-)-α-pinene	17.3	16.57
(+)-α-pinene	36.49	37.3	(-)-camphene	28.5	27.9
(-)-β-pinene	26.0	25.2	(-)-sabinene	7.2	6.45
			(-)-β-pinene	17.6	16.74
			(-)-bornyl acetate	29.4	24.28
					others: 7.96

	Bole volatiles			Foliage volatiles	
Compound[a]	Actual composition (%)	Realised composition (%)	Compound	Actual composition (%)	Realised composition (%)
lodgepole pine (LP)					
(-)-α-pinene	5.94	5.36	(-)-α-pinene	8.4	7.0
(-)-β-pinene	19.15	17.31	(-)-β-pinene	51.3	44.6
(+)-3-carene	6.98	11.65	(-)-β-phellandrene	40.3	30.37
(-)-β-phellandrene	60.73	46.79			(+)-3-carene: 7.4
(-)-limonene	7.19	6.03 others: 12.84			others: 10.56
Engelmann spruce (ESP)					
(-)-α-pinene	39.4	35.55	(-)-α-pinene	13.3	14.29
(+)-α-pinene	9.29	10.27	(-)-camphene	17.0	16.66
(-)-β-pinene	29.46	25.58	myrcene	30.6	25.16
(+)-3-carene	9.94	12.60	(-)-β-phellandrene	8.2	7.07
(-)-β-phellandrene	11.9	9.13	(-)-limonene	7.3	7.09
		others: 6.87	terpinolene	15.5	13.99
			(-)-bornyl acetate	8.1	6.06
					others: 9.32

	Bole volatiles			Foliage volatiles	
Compound[a]	Actual composition (%)	Realised composition (%)	Compound	Actual composition (%)	Realised composition (%)
interior spruce (ISP)					
(-)-α-pinene	35.95	34.94	(-)-α-pinene	11.7	10.2
(+)-α-pinene	21.09	18.89	(-)-camphene	20.1	19.05
(-)-β-pinene	33.0	32.23	(+)-camphene	6.2	5.8
(+)-3-carene	9.96	9.46	myrcene	14.1	12.1
		others: 4.48	(-)-limonene	8.8	8.68
			γ-terpinene	6.7	6.2
			terpinolene	6.9	7.07
			(-)-bornyl acetate	25.5	23.38
					others: 7.52
white spruce (WSP)					
(-)-α-pinene	25.6	22.74	(+)-α-pinene	14.3	14.54
(+)-α-pinene	49.0	50.24	(+)-camphene	25.5	25.11
(-)-β-pinene	15.3	15.7	myrcene	11.0	10.4
(+)-3-carene	10.06	8.85	(-)-limonene	12.8	13.62
		others: 2.47	(-)-bornyl acetate	36.4	33.99
					others: 2.36

	Bole volatiles			Foliage volatiles	
Compound[a]	Actual composition (%)	Realised composition (%)	Compound	Actual composition (%)	Realised composition (%)
interior fir (IF)					
(-)-α-pinene	10.2	9.8	(-)-α-pinene	5.3	4.88
(-)-β-pinene	26.87	24.16	(-)-camphene	9.2	8.06
(+)-3-carene	10.16	8.55	(-)-β-pinene	18.8	17.6
(-)-β-phellandrene	43.33	34.7	(-)-β-phellandrene	35.4	28.39
(-)-limonene	9.45	6.76	(-)-limonene	14.6	12.47
		others: 16.03	(-)-bornyl acetate	16.8	11.93
					others: 16.66

[a]Blends are designated by acronyms used in Figures 5.1-5.7. Three blends of interior spruce (ISP) were made up corresponding to the proximity of hybrid population to the range of pure Engelmann (ESP) and white spruce (WSP). Thus blends representing a population near Princeton are designated ESP, blends representing a population near Prince George are designated WSP, and blends representing populations from 100 Mile House and Kamloops, midway between the other two locations are designated ISP.

in 250 mL portions with commercial bleach to remove odourous sulphur compounds. The turpentine was dried with calcium chloride, filtered, and distilled as above with the distillate collected in one portion. Rectified turpentine was fractionated through a 20 cm Dufton column fitted with a total reflux, partial take-off head at 40 mm Hg. Fractions were taken off and analysed by gas chromatography. Fractions containing 60 – 65 % (-)-β-phellandrene (bp. 89°C) were combined and used in experiments. Because the impurities in fractions enriched with (-)-sabinene or (-)-β-phellandrene were mostly pinenes and terpinolene, which are normal resin constituents, blends were prepared by mixing of fractions with purchased materials to achieve as close a ratio of compounds as possible, to the composition of the desired blend (Table 5.2.). For experimental testing, synthetic blends were released from polyethylene bottles (Table 5.3.). The volatile composition of the headspace outside the enclosed bottle was sampled from a closed glass chamber with a syringe, and gas chromatographed to confirm that the composition of released volatiles reflected that of the blend.

Field trapping

Eighteen experiments evaluating the attraction of the three species of beetles to conifer bark and foliage blends were conducted in various locations in British Columbia in the summers of 2001 and 2002. The volatile spectra of Douglas-fir on the coast varied significantly from those in the interior (Chapter 4). Hence, coastal experiments for *D. pseudotsugae* were run at the University of British Columbia's Malcolm Knapp Research Forest, Maple Ridge, B.C., from 10 to 17 May, 2001 and 7 to 21 May, 2002,

Table 5.3. Chemicals used in field trapping experiments, release rates and devices.

Chemical	Purity (%)	Source	Release device	Release rate (mg/24 h) and temperature[a]
Attractive lures				
D. pseudotsugae				
(±)-frontalin	>99	Phero Tech Inc.	0.4 mL polyethylene vial	2.6 at 23°C*
(±)-MCOL	98		bubble cap	2.0 at 20°C*
D. ponderosae		Phero Tech Inc.		
myrcene	93		20 mL low density polyethylene bottle	95 at 23°C*
(±)-*exo*-brevicomin	99		flexlure	0.28 at 20°C*
82% (-)-*trans*-verbenol	>95		bubble cap	1.5 at 20°C*
D. rufipennis		Phero Tech Inc.		
(-)- α-pinene	>99		1.5 mL polypropylene vial	1.5 at 20°C*
(±)-frontalin	>99		0.4 mL polyethylene vial	2.6 at 23°C*
(±)-MCOL	98		bubble cap	2.0 at 20°C*
Synthetic conifer blends (Compounds listed in Table 5.1 were mixed in ratios from Table 5.2)		Mixed in the laboratory	20 mL low density polyethylene bottle containing 2.5 mL of a blend	140 at 25°C

[a] Release rate determined by Phero Tech Inc., Delta, B.C. if followed by asterisk. Otherwise determined by DSP. Commercial lures for use in traps contain host monoterpenes.

and interior experiments were run at the university's Alex Fraser Research Forest near William's Lake, B.C., from 15 to 27 June, 2001. *Dendroctonus ponderosae* were trapped from 8 August to 17 September, 2001 on Opax Mountain, 25 km northwest of Kamloops, and *D. rufipennis* were trapped from 26 June to 18 July, 2002 on Blue Jay and Augier Roads, 30 km north of Burns Lake.

Three blends of interior spruce volatiles (Chapter 4) were used, corresponding to the most predominant expected influence of Engelmann and white spruce on the hybrid population, and the nearest geographic location of a trapping experiment. Thus, the blends from Princeton (ESP), 100 Mile House (ISP) and Prince George (WSP) (Table 5.2.) were used for experiments near: Maple Ridge (ESP); Kamloops, Lumby and William's Lake (ISP), and Burns Lake (WSP), respectively. All experiments employed 12-unit multiple funnel traps (Lindgren, 1983), set up in randomised complete blocks, with $\geq$ 15 m between traps.

For each species, one set of experiments was conducted to determine whether there was primary attraction to host volatiles, and a second set to evaluate if beetles discriminated among the odours of the four different species of conifers. In 2001, the test of primary attraction was performed with three experiments each for *D. pseudotsugae* and *D. ponderosae,* which tested host bole and foliage volatiles alone and together. Treatments were: 1) an attractive pheromone bait (positive control), 2) unbaited trap (negative control), 3) conifer volatiles, and 4) bait plus conifer volatiles. For *D. rufipennis* in 2002, positive controls were not included. Instead, baited monitoring traps were set up at the first, 20^{th}, 40^{th} and 60^{th} trap positions to monitor if flight had occurred

in the area. In addition, bole and foliage volatiles were tested alone and together in the same experiment. Traps on either side of a baited monitoring trap were no more effective than other experimental traps.

For host discrimination experiments, bole and foliage volatiles of all conifer species were tested in combination with the attractive bait in two separate experiments for all species. The attractive baits alone (Table 5.3.) were used as positive controls and unbaited traps as negative controls. For *D. ponderosae* and *D. rufipennis*, myrcene and α-pinene, respectively, are used in combination with aggregation pheromones as part of the commercial trap bait. Therefore, the commercial bait was included as an additional treatment to compare the effect of the conifer blends relative to the synergistic effect of a single monoterpene. The number of replicates ranged from 6-15, as some were lost due to interference from wind, bears and cattle. Captured beetles were frozen until they were identified, sexed (Lyon, 1958; Jantz & Johnsey, 1964) and counted. All data were transformed by $\log_{10}(x+1)$ to meet the assumptions of normality and homoscedasticity, and analysed by ANOVA (Proc ANOVA), with treatment and block as main effects, and the Ryan-Einot-Gabriel-Welsh multiple range test (Day & Quinn, 1989; SAS Institute Inc., 1990), to determine 1) if there were differences in numbers of beetles captured in traps with host volatiles, compared to each of the other treatments in the primary attraction experiments and 2) there were differences in numbers of beetles captured in traps with host *versus* nonhost volatiles in the host discrimination experiments. In all cases, $\alpha = 0.05$.

5.3. Results

Coastal *D. pseudotsugae*

More coastal *D. pseudotsugae* of both sexes were caught in traps baited with the aggregation pheromone bait (frontalin+MCOL) and bole volatiles than in traps with the bait alone (Figure 5.1.A) (males: F = 54.43, df = 3,42, $P < 0.0001$; females: F =20, df = 3,42, $P < 0.0001$). The bait attracted significantly more males, but not females, than the bole volatiles alone. There were no differences between the numbers of either sex captured in unbaited traps and traps baited with bole volatiles. Foliage volatiles in combination with the bait attracted more males than the pheromone bait, which was more attractive than the foliage volatiles alone or unbaited controls (Figure 5.1.B) (F = 16.02, df = 3,36, $P < 0.0001$). More females were captured in traps with the bait and foliage volatiles than in unbaited traps (F =3.84, df = 3,36, $P = 0.0175$), but there was no difference in response to the bait or foliage volatiles alone, compared to unbaited traps. Catches of both sexes in traps with both bole and foliage volatiles combined with the bait, were greater than in traps releasing host volatiles alone, but not different from traps with the bait alone or unbaited traps (Figure 5.1.C) (males: F = 58.21, df = 3,33, $P < 0.0001$; females: F =17.2, df = 3,33, $P < 0.0001$).

Fewer beetles of both sexes were caught in traps with the pheromone bait plus bole volatiles of lodgepole pine and interior fir, than in traps baited with bole volatiles of coastal Douglas-fir, interior Douglas-fir, or interior (Engelmann) spruce (Figure 5.2.A) (males: F = 47.42, df = 6,78, $P < 0.0001$; females: F = 38.64, df = 6,78, $P < 0.0001$). Males were significantly less attracted to the pheromone bait with foliage volatiles of

Figure 5.1. Numbers of coastal *D. pseudotsugae* captured in field trapping experiments with volatiles of coastal Douglas-fir (CDF): (A) bole volatiles (n=15), (B) foliage volatiles (n=12), and (C) bole and foliage volatiles (n=12). Treatments are ordered for female (the first attacking sex) trap catch. Bars for each sex and experiment with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

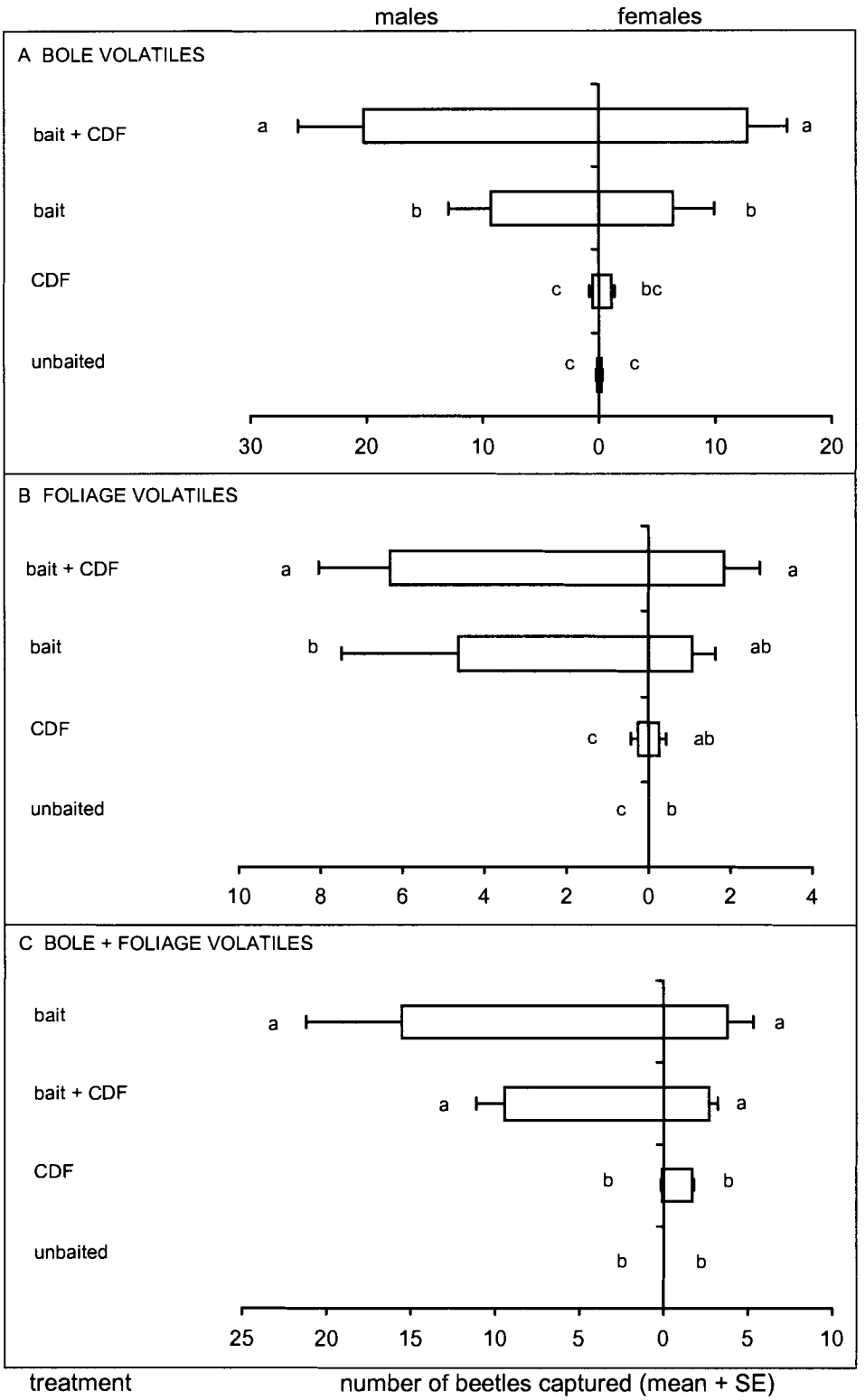
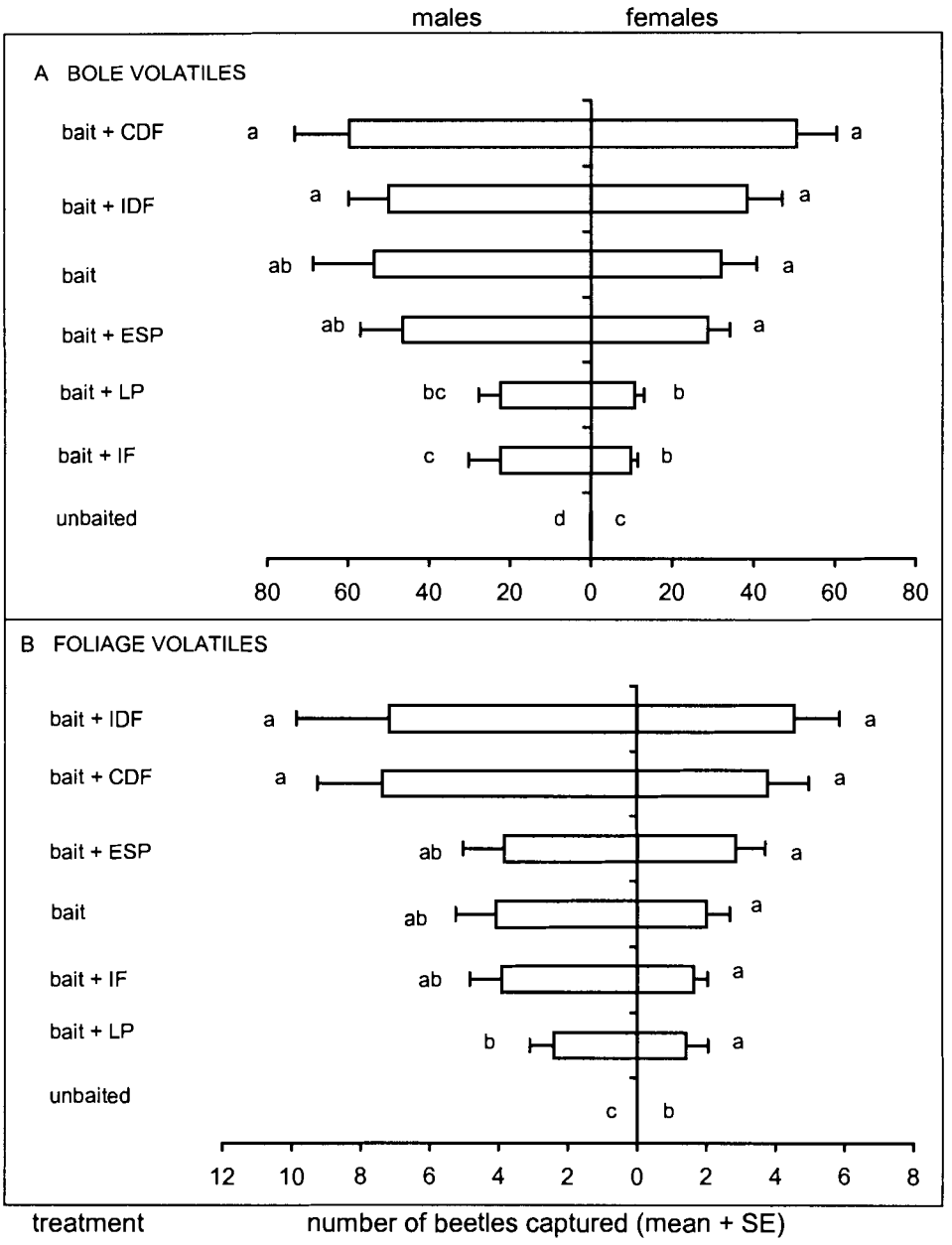

males
females
A BOLE VOLATILES
bait + CDF
bait
CDF
unbaited
a
a
b
b
c
bc
c
c
30
20
10
0
10
20
B FOLIAGE VOLATILES
bait + CDF
bait
CDF
unbaited
a
a
b
ab
c
ab
c
b
10
8
6
4
2
0
2
4
C BOLE + FOLIAGE VOLATILES
bait
bait + CDF
CDF
unbaited
a
a
a
a
b
b
b
b
25
20
15
10
5
0
5
10
treatment
number of beetles captured (mean + SE)

Figure 5.2. Numbers of coastal *D. pseudotsugae* captured in field trapping experiments with volatiles of four species of conifers: (A) bole volatiles (n=14), and (B) foliage volatiles (n=12). Acronyms for source populations of trees given in Table 5.2. Treatments are ordered for female (the first attacking sex) trap catch. Bars for each sex and experiment with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.


males
females
A BOLE VOLATILES
bait + CDF
bait + IDF
bait
bait + ESP
bait + LP
bait + IF
unbaited
a
a
a
a
ab
a
ab
a
bc
b
c
b
d
c
80
60
40
20
0
20
40
60
80
B FOLIAGE VOLATILES
bait + IDF
bait + CDF
bait + ESP
bait
bait + IF
bait + LP
unbaited
a
a
a
a
ab
a
ab
a
ab
a
b
a
c
b
12
10
8
6
4
2
0
2
4
6
8
treatment
number of beetles captured (mean + SE)

lodgepole pine than to the bait with volatiles of interior or coastal Douglas-fir (Figure 5.2.B) ($F = 10.22$, $df = 6,64$, $P < 0.0001$). In contrast to experiments with bole and foliage volatiles (Figure 5.1), no bole or foliage volatile blend enhanced catches of either sex over those in traps with the pheromone bait alone. In both experiments, unbaited control traps caught fewer beetles of either sex than any experimental trap (Figures 5.2.A,B).

Interior *D. pseudotsugae*

The only enhancement of catches of interior *D. pseudotsugae* over those in traps with the pheromone bait alone was for females with bait plus bole volatiles (Figure 5.3. A) (males: $F = 156.46$, $df = 3,40$, $P < 0.0001$; females: $F = 57.28$, $df = 3,40$, $P < 0.0001$) (Figure 5.3.B) (males: $F = 216.68$, $df = 3,24$, $P < 0.0001$; females: $F = 111.50$, $df = 3,24$, $P < 0.0001$). More males and females were caught in traps with bole volatiles alone than in unbaited control traps (Figure 5.3.A). In contrast to the experiment that tested bole + foliage volatiles (Figure 5.1.C), traps baited with bole and foliage volatiles in combination with the pheromone bait captured more females than traps with the bait alone, although the number of males caught did not differ significantly between the two treatments (Figure 5.3.C) (males: $F = 22.35$, $df = 3,41$, $P < 0.0001$; females: $F = 32.84$, $df = 3,41$, $P < 0.0001$). Catches in traps with bole and foliage volatiles combined were no greater than in unbaited control traps.

Females were less attracted to traps with the pheromone bait plus the bole volatiles of interior fir and lodgepole pine than to the bait and volatiles of coastal Douglas-fir (Figure 5.4.A) ($F = 35.41$, $df = 6,82$, $P < 0.0001$), but did not differentiate

Figure 5.3. Numbers of interior *D. pseudotsugae* captured in field trapping experiments with volatiles of interior Douglas-fir (IDF): (A) bole volatiles (n=15), (B) foliage volatiles (n=9) and (C) bole and foliage volatiles (n=15). Treatments are ordered for female (the first attacking sex) trap catch. Bars for each sex and experiment with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

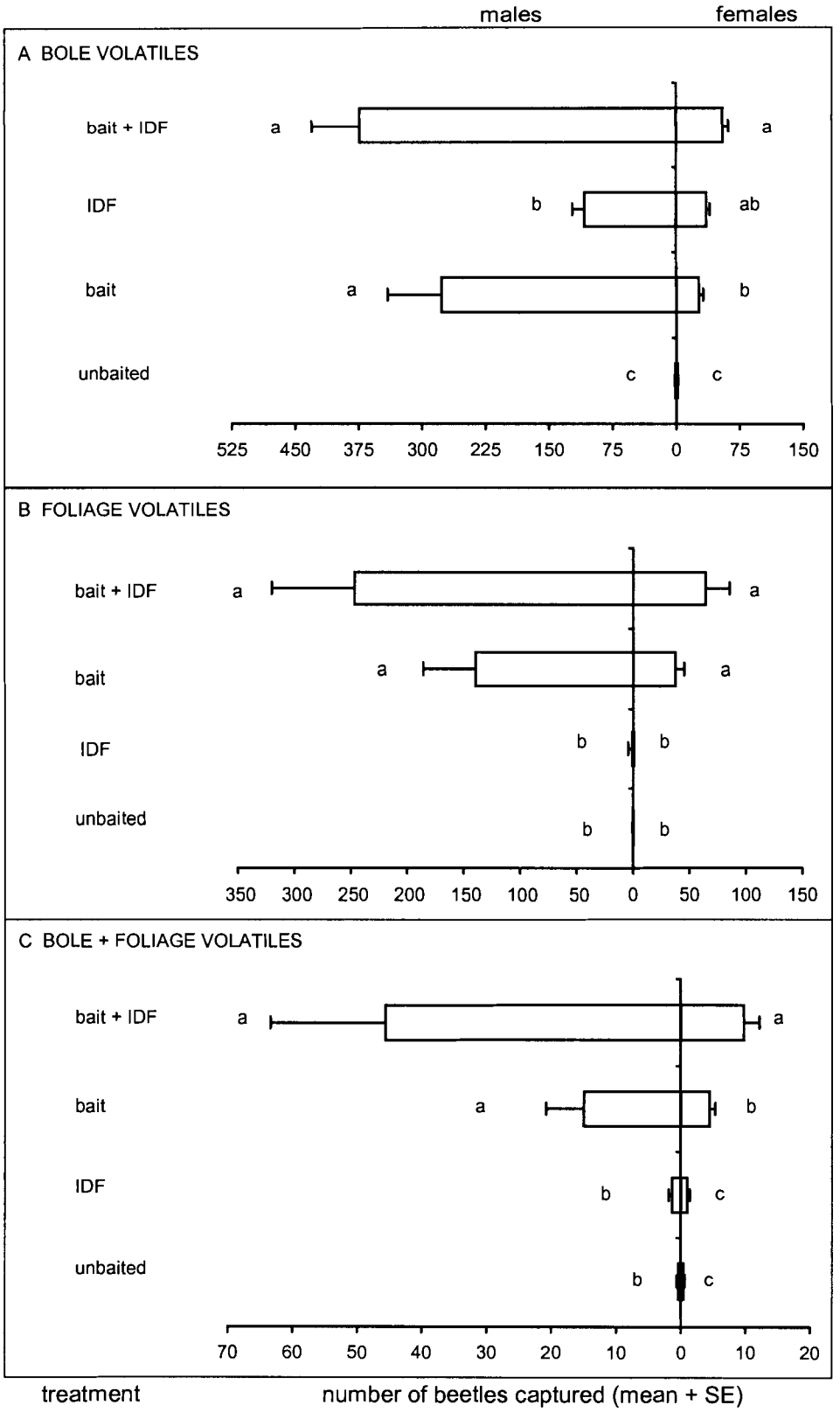
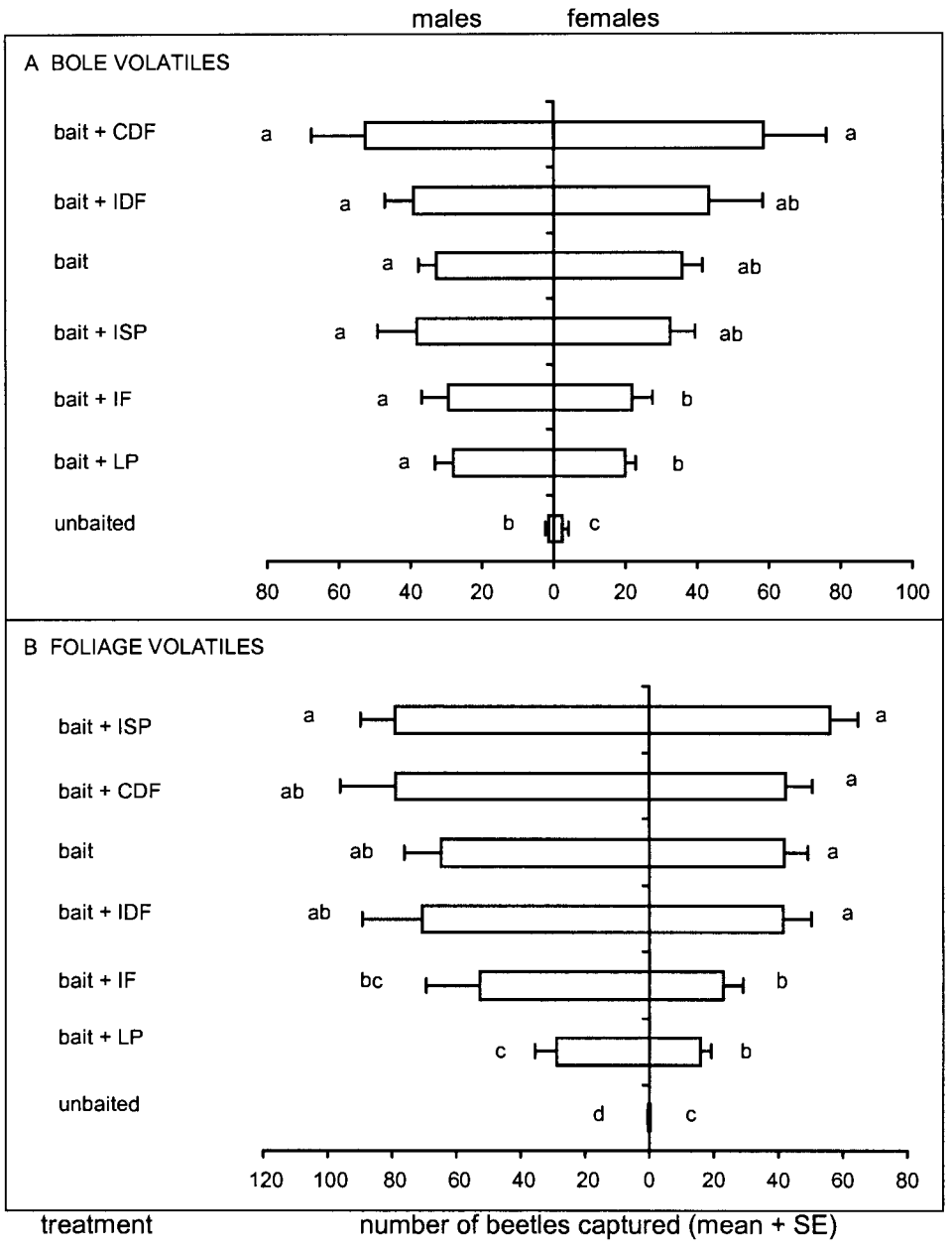

males
females
A BOLE VOLATILES
bait + IDF
IDF
bait
unbaited
a
a
b
ab
a
b
c
c
525 450 375 300 225 150 75 0 75 150
B FOLIAGE VOLATILES
bait + IDF
bait
IDF
unbaited
a
a
a
a
b
b
b
b
350 300 250 200 150 100 50 0 50 100 150
C BOLE + FOLIAGE VOLATILES
bait + IDF
bait
IDF
unbaited
a
a
a
b
b
c
b
c
70 60 50 40 30 20 10 0 10 20
treatment
number of beetles captured (mean + SE)

Figure 5.4. Numbers of interior *D. pseudotsugae* captured in field trapping experiments with volatiles of four species of conifers: (A) bole volatiles (n=15), and (B) foliage volatiles (n=15). Acronyms for source populations of trees given in Table 5.2. Treatments are ordered for female (the first attacking sex) trap catch. Bars for each sex and experiment with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.


males
females
A BOLE VOLATILES
bait + CDF
bait + IDF
bait
bait + ISP
bait + IF
bait + LP
unbaited
a
a
a
ab
a
ab
a
ab
a
b
a
b
b
c
80 60 40 20 0 20 40 60 80 100
B FOLIAGE VOLATILES
bait + ISP
bait + CDF
bait
bait + IDF
bait + IF
bait + LP
unbaited
a
a
ab
a
ab
a
ab
a
bc
b
c
b
d
c
120 100 80 60 40 20 0 20 40 60 80
treatment
number of beetles captured (mean + SE)

significantly among other blends. In the experiment with foliage volatiles, males were less attracted to the pheromone bait plus foliage volatiles of lodgepole pine than to the other blends, except the bait plus interior fir foliage volatiles (Figure 5.4.B) ($F = 51.79$, df = 6,84, $P < 0.0001$). Females were significantly less attracted to traps with the bait plus interior fir or lodgepole pine foliage volatiles compared to all other blends ($F = 55.69$, df = 6,84, $P < 0.0001$).

D. ponderosae

The commercial trap bait (myrcene plus the aggregation pheromones *trans*-verbenol and *exo*-brevicomin) caught significantly more *D. ponderosae* of both sexes than traps with any other treatment (Figure 5.5.A) (males: $F = 29.71$, df = 4,42, $P < 0.0001$; females: $F = 25.31$, df = 4,42, $P < 0.0001$) including the bait plus lodgepole pine bole volatiles, which were no more attractive than the bait alone. The bole volatiles alone were not attractive. Similar results were obtained with foliage volatiles except that traps with the bait plus foliage volatiles caught more beetles than the bait alone (Figure 5.5.B) (males: $F = 70.31$, df = 4,43, $P < 0.0001$; females: $F = 47.13$, df = 4,43, $P < 0.0001$). Combining the bait with both bole and foliage volatiles, did not improve trap catches over those in traps with the bait alone (Figure 5.5.C) (males: $F = 42.69$, df = 4,54, $P < 0.0001$; females: $F = 46.55$, df = 4,54, $P < 0.0001$). Again, myrcene plus the bait was the most attractive stimulus for both sexes.

Males were more attracted to the pheromone bait plus myrcene than to any other treatment involving bole volatiles (Figure 5.6.A) ($F = 11.76$, df = 6,83, $P < 0.0001$),

Figure 5.5. Numbers of *D. ponderosae* captured in field trapping experiments with volatiles of lodgepole pine (LP): (A) bole volatiles (n=12) (B) foliage volatiles (n=12) and (C) both bole and foliage volatiles (n=15). Treatments are ordered for female (the first attacking sex) trap catch. Bars for each sex and experiment with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

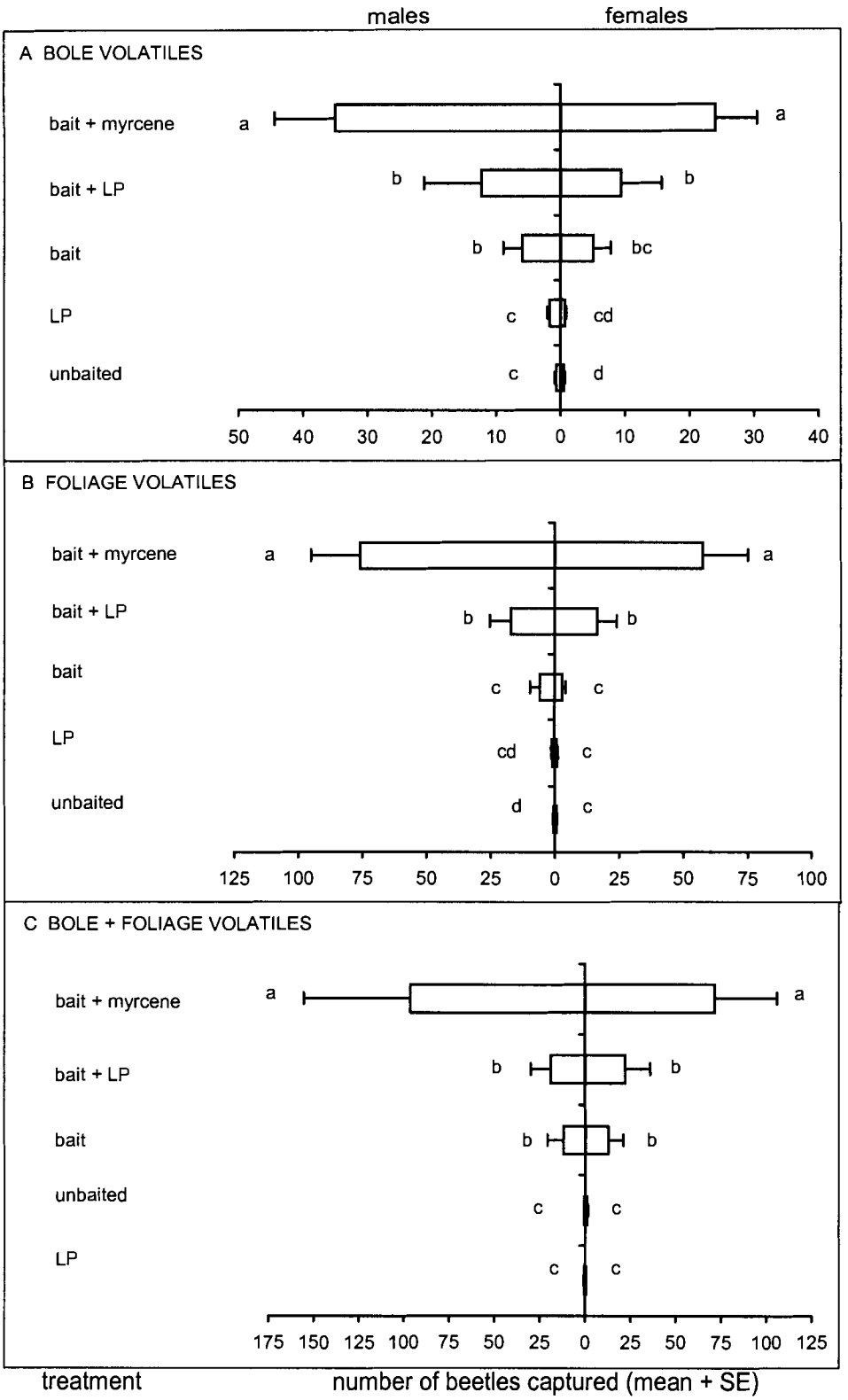
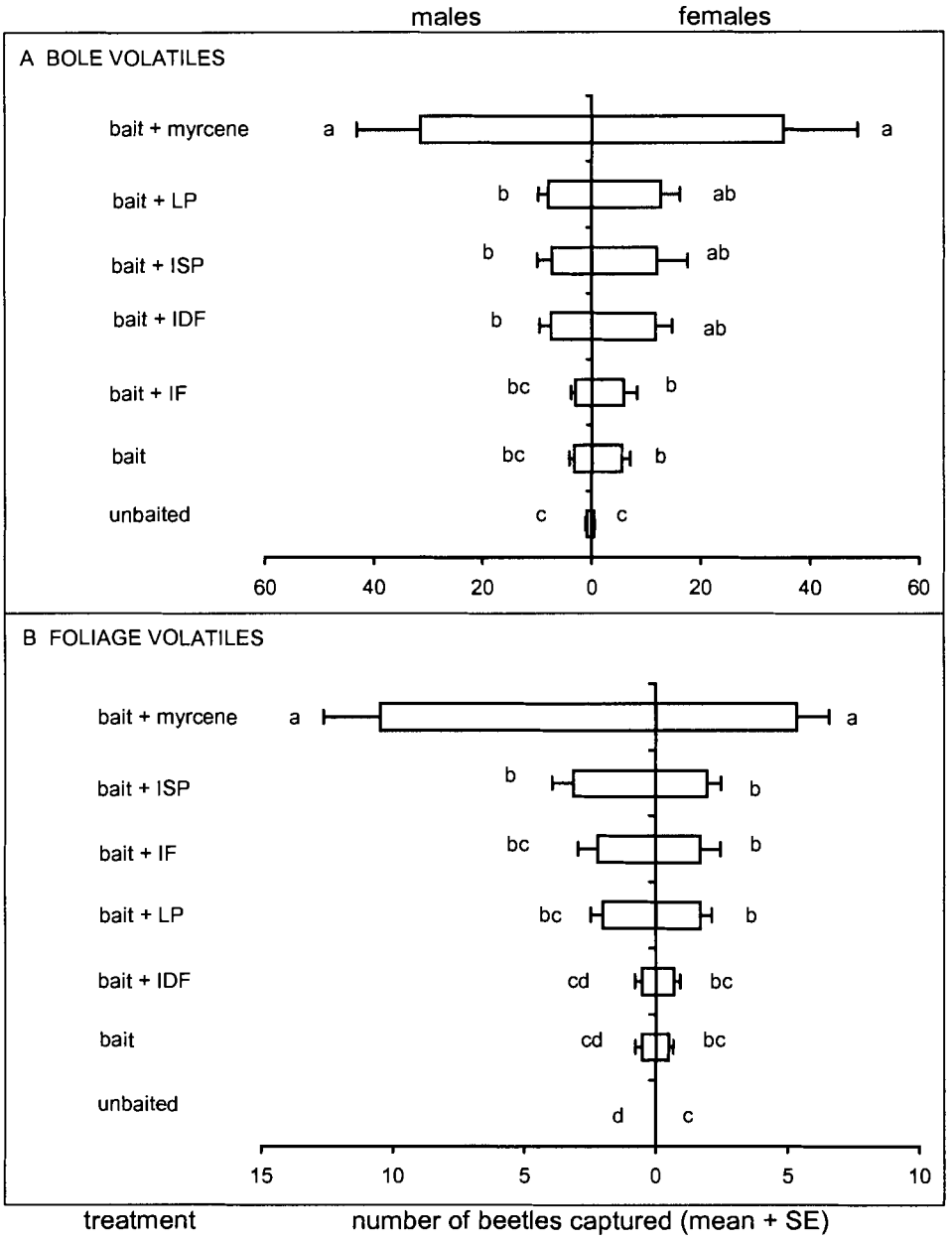

males
females
A BOLE VOLATILES
bait + myrcene
bait + LP
bait
LP
unbaited
a
a
b
b
b
bc
c
cd
c
d
50 40 30 20 10 0 10 20 30 40
B FOLIAGE VOLATILES
bait + myrcene
bait + LP
bait
LP
unbaited
a
a
b
b
c
c
cd
c
d
c
125 100 75 50 25 0 25 50 75 100
C BOLE + FOLIAGE VOLATILES
bait + myrcene
bait + LP
bait
unbaited
LP
a
a
b
b
b
b
c
c
c
c
175 150 125 100 75 50 25 0 25 50 75 100 125
treatment
number of beetles captured (mean + SE)

Figure 5.6. Numbers of *D. ponderosae* captured in field trapping experiments with volatiles from four species of conifers: (A) bole (n=15), and (B) foliage (n=15) volatiles. Acronyms for source populations of trees given in Table 5.2. Treatments are ordered for female (the first attacking sex) trap catch. Bars for each sex and experiment with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

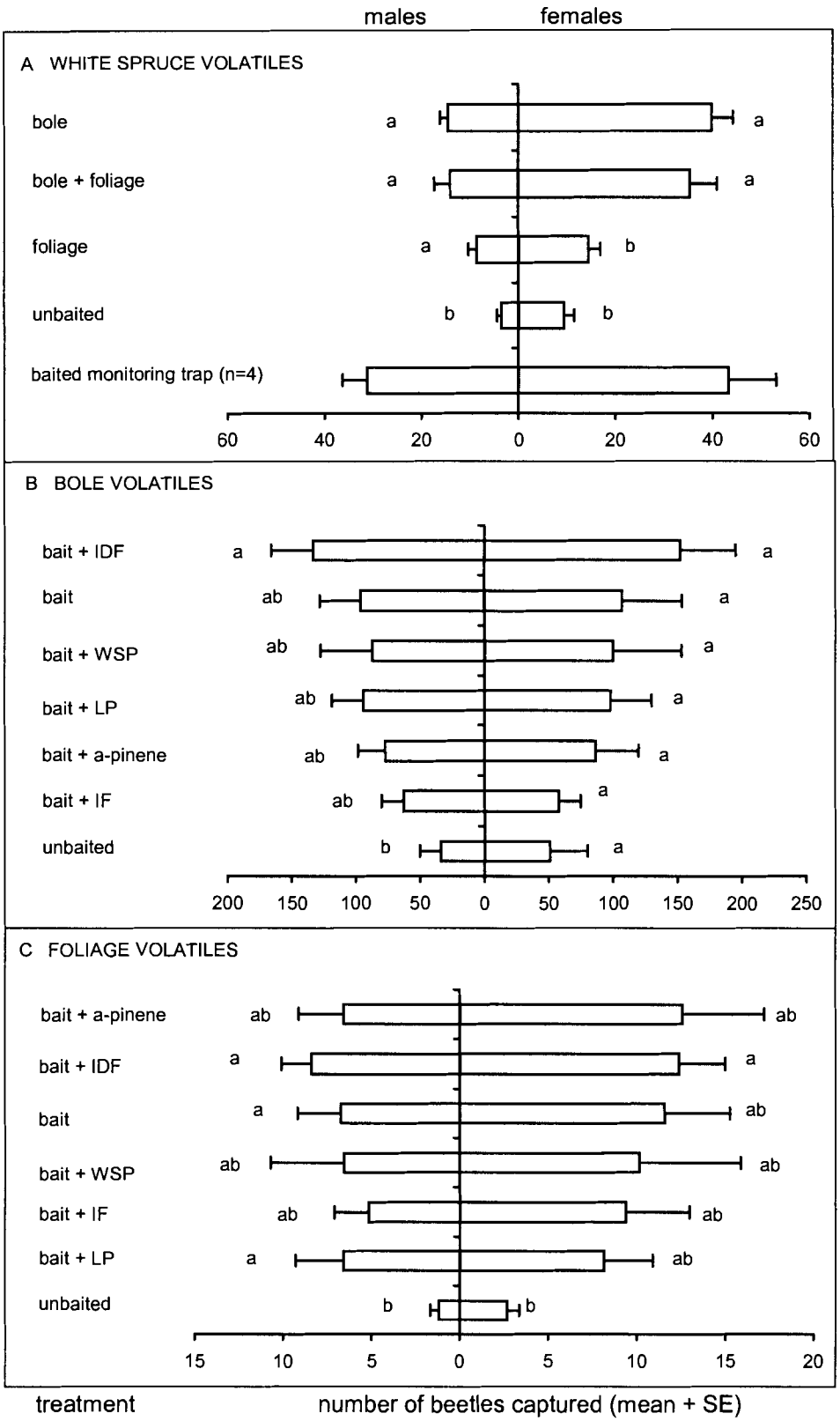

males
females
A BOLE VOLATILES
bait + myrcene
bait + LP
bait + ISP
bait + IDF
bait + IF
bait
unbaited
a
a
b
ab
b
ab
b
ab
bc
b
bc
b
c
c
60
40
20
0
20
40
60
B FOLIAGE VOLATILES
bait + myrcene
bait + ISP
bait + IF
bait + LP
bait + IDF
bait
unbaited
a
a
b
b
bc
b
bc
b
cd
bc
cd
bc
d
c
15
10
5
0
5
10
treatment
number of beetles captured (mean + SE)

while females were less attracted to the bait plus bole volatiles of interior fir and the bait alone than to the bait plus myrcene ($F = 12.63$, $df = 6,83$, $P < 0.0001$). Similarly, more *D. ponderosae* of both sexes were caught in traps containing the bait plus myrcene than in traps with any foliage volatile blend (Figure 5.6.B) (males: $F = 20.47$, $df = 6,83$, $P < 0.0001$; females: $F = 10.51$, $df = 6,83$, $P < 0.0001$). Interior spruce foliage volatiles (but not those of lodgepole pine) in combination with the pheromone bait enhanced the catch of males over that of the bait alone. No other foliage volatile blend had such an effect on either sex.

D. rufipennis

Male *D. rufipennis* were significantly more attracted to traps baited with white spruce bole, foliage, and bole plus foliage volatiles than to unbaited control traps (Figure 5.7.A) ($F = 8.92$, $df = 3,38$, $P < 0.0001$). Females responded similarly, but not to foliage volatiles alone ($F = 18.22$, $df = 3,38$, $P < 0.0001$). Males were more attracted to traps with the pheromone plus interior Douglas-fir bole volatiles than unbaited control traps (Figure 5.7.B) ($F = 3.08$, $df = 6,47$, $P < 0.0127$) but females did not discriminate among treatments ($F = 2.16$, $df = 6,47$, $P < 0.0638$). Neither sex discriminated among the foliage volatiles of the four species of conifers, or the attractive baits (Figure 5.7.C) (males: $F = 2.93$, $df = 6,35$, $P < 0.0127$; females: $F = 1.78$, $df = 6,35$, $P < 0.1323$). Males were more attracted to treatment traps with the pheromone bait alone or in combination with interior Douglas-fir or lodgepole pine foliage volatiles, while females responded only to traps with the bait plus interior Douglas-fir volatiles.

Figure 5.7. Numbers of *D. rufipennis* captured in field trapping experiments with (A) bole and foliage volatiles (n=14) of white spruce (WSP), and (B) bole (n=9), and (C) foliage (n=7) volatiles from four species of conifers. Acronyms for source populations of trees given in Table 5.2. Treatments are ordered for female (the first attacking sex) trap catch. Bars for each sex and experiment with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.


males
females
A WHITE SPRUCE VOLATILES
bole
bole + foliage
foliage
unbaited
baited monitoring trap (n=4)
a
a
a
a
a
b
b
b
60
40
20
0
20
40
60
B BOLE VOLATILES
bait + IDF
bait
bait + WSP
bait + LP
bait + a-pinene
bait + IF
unbaited
a
ab
ab
ab
ab
ab
b
a
a
a
a
a
a
200
150
100
50
0
50
100
150
200
250
C FOLIAGE VOLATILES
bait + a-pinene
bait + IDF
bait
bait + WSP
bait + IF
bait + LP
unbaited
ab
a
a
ab
ab
a
b
ab
a
ab
ab
ab
ab
b
15
10
5
0
5
10
15
20
treatment
number of beetles captured (mean + SE)

5.4. Discussion

The primary attraction hypothesis was proposed by Person (1931), when he observed that the western pine beetle, *D. brevicomis*, was attracted to weakened *P. ponderosa*. In this study, primary attraction to host volatiles was observed in two instances, the response of interior *D. pseudotsugae* to the bole volatiles of interior Douglas-fir (Figure 5.3.A) and that of *D. rufipennis* to volatiles from both bark and foliage of white spruce (Figure 5.7.A). These results support those of McMullen & Atkins (1962), who did not detect evidence of random landing during the host selection by *D. pseudotsugae*, and (Rudinsky, 1966a), who found *D. pseudotsugae* to be attracted to the resin components α-pinene, camphene and limonene. They also support Moeck's (1978) observation that *D. rufipennis* oriented preferentially to cages containing cut logs of white spruce compared to empty ones. The existence of primary attraction can thus be confirmed for *D. pseudotsugae* and *D. rufipennis*. My results did not confirm primary attraction in *D. ponderosae,* supporting observations of random landing by this species (Hynum & Berryman, 1980), but opposing observations of orientation to cages containing bark and wood of lodgepole pine (Moeck & Simmons, 1991) and preferential landing on fire-scarred and moribund lodgepole pines (Gara *et al.*, 1984).

Primary attraction to host volatiles would increase bark beetle fitness by reducing mortality during dispersal (Gries *et al.*, 1989). However, for species with strong aggregation pheromones, only a small proportion of dispersing beetles would need to detect host trees before landing, particularly at high population densities, as the rest of the

population can exhibit secondary attraction, the orientation to host volatiles in combination with aggregation pheromones produced by pioneer beetles (Wood, 1972). Observations of weak or absent primary attraction in aggressive species support this hypothesis (Byers, 1989, 1995, 1996), e.g. no difference in landing rates by *D. ponderosae* on live and dead lodgepole pines, or on hosts and nonhosts (Hynum & Berryman, 1980), and equal landing rates by *D. brevicomis*, on healthy and stressed ponderosa pine (Moeck *et al.*, 1981). Fir engravers, *Scolytus ventralis* LeConte, apparently colonised their host, *Abies grandis* (Dougl. ex D. Don) Lindl., at random (Berryman & Ashraf, 1970), but the absence of aggregation pheromones and the strong attraction to host volatiles suggest that host selection by this aggressive species occurs by primary attraction (Macias-Samano *et al.*, 1998a,b).

Less aggressive species of bark beetles, that preferentially attack moribund trees rather than healthy trees, and secondary species that attack only moribund trees, are more prone to being strongly attracted to host volatiles, ethanol or both (Kohnle, 1985; Klimetzek *et al.*, 1986; Schroeder, 1988; Schroeder & Lindelow, 1989) as is evident in *D. pseudotsugae* and *D. rufipennis* from my experiments. Nine species including *D. pseudotsugae* and *Dryocoetes autographus* Swaine, were attracted to Douglas-fir oleoresin and its monoterpene components (Rudinsky, 1966c). The red turpentine beetle, *Dendroctonus valens* LeConte, was caught in traps baited with cut logs and oleoresin from ponderosa pine (Vité & Gara, 1962), and host monoterpenes (+)-α-pinene, (-)-β-pinene, and (+)-3-carene (Hobson *et al.*, 1993). The pine shoot beetle, *T. piniperda* was strongly attracted to monoterpenes released from host Scots pine logs (Byers *et al.*,

1985). In the absence of pheromones, *I. latidens* and *Hylastes gracilis* LeConte, were attracted to girdled, but not intact lodgepole pine (Miller *et al.*, 1986). Traps baited with bolts of Scots pine, and Norway spruce, *P. abies*, caught *T. piniperda*, *Hylastes brunneus* Erichson, and *P. bidentatus,* while *Hylastes cunicularius* Erichson preferred Norway spruce (Tunset *et al.*, 1993). Primary attraction and pre-landing discrimination among traps baited with billets of *B. pubescens*, *Alnus incana* (L.) Moench or *P. sylvestris*, occurred in *T.piniperda*, *H. brunneus*, and *Pityogenes quadricens* (Hartig) (Brattli *et al.*, 1998).

Only coastal and interior *D. pseudotsugae* demonstrated discrimination among volatile blends from different conifers, and even this discrimination was incomplete (Figures 5.2., 5.4.). Beetles were significantly less attracted to pheromone baits combined with volatile blends of lodgepole pine and interior fir than they were to baits alone. Relatively low α-pinene and correspondingly high β-phellandrene content in the volatiles of lodgepole pine and interior fir, compared to those of Douglas-fir and spruce (Chapter 4) could be the basis for this discrimination. Although coastal and interior Douglas-fir differed significantly in their quantitative chemical profiles (Chapter 4), neither coastal nor interior beetles were able to distinguish between them. This is probably because beetles are attracted to monoterpene profiles with high levels of α-pinene compared to β-phellandrene that occur in both coastal and interior Douglas-fir. Heikkenen & Hrutfiord (1965), proposed that *D. pseudotsugae* locate hosts in response to volatile stimuli and found that ratios and concentrations of terpenes influenced attraction of beetles. α-Pinene was attractive and β-pinene highly repellent in their study. However, my study indicates

that *D. pseudotsugae* discriminated among certain species of conifers (they avoided volatiles from lodgepole pine and subalpine fir), but not between coastal and interior Douglas-fir. When given a choice among Douglas-fir, western red cedar, *Thuja plicata* Donn, western white pine, *P. monticola,* and western hemlock, *Tsuga heterophylla* (Raf.) Sarg., *D. pseudotsugae* was attracted only to odours from Douglas-fir (Chapman, 1963).

Dendroctonus ponderosae did not discriminate significantly among the volatiles blends from different conifers. Stronger attraction to the pheromone bait plus myrcene than to any conifer volatile blend plus pheromone is an enigma. Even though myrcene constitutes only 2.6 % and 1.1 %, respectively, of bole and foliage volatiles of lodgepole pine (Chapter 4), it is the strongest known synergist to the aggregation pheromones of *D. ponderosae* in British Columbia (Conn *et al.*, 1983; Borden *et al.*, 1983, 1987a). The only apparent discrimination among conifer volatile blends occurred when males responded in lower numbers to the pheromone bait in combination with foliage volatiles from interior Douglas-fir compared to volatiles of interior spruce (Figure 5.6.B) which contained 12% myrcene (Table 5.2.).

Like *D. ponderosae*, *D. rufipennis* also did not discriminate among blends of volatiles from different conifers (Figure 5.7.B,C). Strong attraction of this species to volatiles of white spruce (Figure 5.7. A) (Moeck, 1978), equal landing rates of females but not males on pheromone-baited hosts and nonhosts, and their failure to initiate attack on nonhosts (Chapter 2), suggests that host selection in this species may involve long range attraction to host volatiles such as α-pinene, and rejection of unsuitable trees at short range, either just before or immediately after landing.

Few other studies have investigated how bark beetles distinguish among sympatric species of conifers, and none has tested synthetic blends that attempt to reconstitute the volatile profiles of naturally occurring sympatric hosts and nonhosts. *Ips paraconfusus* Lanier landed and initiated attack on nonhosts white fir, *Abies concolor* (Gord & Glend) Lindl. ex Hildebr, and preferred ground nonhost phloem with artificial grooves resembling hosts, over ungrooved host phloem (Elkinton & Wood, 1980), suggesting that tactile stimuli, after beetles land on trees, may influence host selection. There is evidence for lack of host discrimination by *D. ponderosae* based on volatile content of conifers (Wood, 1976; Raffa & Berryman, 1982a; Sturgeon & Mitton, 1986). Wood (1976) reported no discrimination between hosts and nonhosts or healthy and susceptible hosts during flight. Raffa & Berryman (1982a) detected no relationship between resistance to *D. ponderosae* and monoterpene composition of lodgepole pine. Sturgeon & Mitton (1986) found that while *D. brevicomis* in California preferred trees with low limonene and high α-pinene content, there were no differences in the means and variances of monoterpene amounts of ponderosa pines in Colorado that survived attack by *D. ponderosae,* compared to unattacked trees in the population. In Chapter 2, I documented equal landing by *D. ponderosae* on pheromone-baited host lodgepole pine trees and nonhost Douglas-fir, as well as initiation of unsuccessful attacks on Douglas-fir, suggesting that reliance on secondary attraction and vigour of attacking beetles, override the need for prelanding olfactory discrimination among potential conifer hosts in this species, whose major host, lodgepole pine, grows predominantly in large monocultures. For other bark beetle species such as *D. pseudotsugae* and *D. rufipennis*, whose hosts

grow in relatively mixed stands, it would be advantageous for pioneer beetles to locate hosts by primary attraction rather than random landing.

6. Repellent semiochemicals from volatiles of bark beetles, for three species of *Dendroctonus*

6.1. Introduction

Interspecific interactions among coniferophagous bark beetles are common when two or more species attack and colonise the same host (Svihra *et al.*, 1980; Rankin & Borden, 1991; Poland & Borden, 1998a,b,c; Ayres *et al.*, 2001). Beetles may be attracted to semiochemicals emitted by heterospecifics and apparently use them to locate hosts which may be rare or patchy in distribution (Poland & Borden, 1994; Savoie *et al.*, 1998; Ayres *et al.*, 2001). Alternatively, they may be repelled, a response that would facilitate resource partitioning, decrease interspecific competition (Byers *et al.*, 1984; Borden *et al.*, 1992; Poland & Borden, 1998a,b,c; Pureswaran *et al.*, 2000; Ayres *et al.*, 2001), and maximise survival of brood.

While several studies document the perception and deterrence of bark beetles to semiochemicals emitted by heterospecifics inhabiting the same host species (Svihra *et al.*, 1980; Light *et al.*, 1983; Rankin & Borden, 1991; Borden *et al.*, 1992; Poland & Borden, 1998a,b,c; Savoie *et al.*, 1998; Pureswaran *et al.*, 2000; Ayres *et al.*, 2001), no studies have investigated whether bark beetles can perceive and avoid semiochemicals produced by heterospecifics that attack nonhost conifers.

In Chapter 3, I used gas chromatographic-electroantennographic detection analyses (GC-EAD) and GC-mass spectrometry (GC-MS) to survey the production and

perception profiles of semiochemicals in four sympatric species of tree-killing bark beetles. I identified nine compounds from the volatiles of the three species of *Dendroctonus* that elicited antennal responses in one or more species for which the behavioural response was unknown. In Chapter 4, I demonstrated that conifers varied significantly in their overall monoterpene profiles. In Chapter 5, I showed that primary attraction to host volatiles occurred in *D. pseudotsugae* and *D. rufipennis*, and that *D. pseudotsugae* responded in lower numbers to traps baited with volatiles of lodgepole pine and interior fir compared to volatiles of Douglas-fir and interior spruce.

In this Chapter, I hypothesised that during the complex process of host selection, bark beetles can avoid nonhost conifers, partly by perceiving and avoiding compounds produced by heterospecifics attacking these nonhosts. I tested each compound identified in Chapter 3, whose behavioural activity was not known, alone and in combination with an attractive pheromone bait. Positive controls included pheromone-baited traps and blank controls included unbaited traps. I report the results of field trapping experiments that determine the behavioural activity of these compounds, and present a list of all identified behaviourally active compounds and their functions in *D. pseudotsugae*, *D. ponderosae*, and *D. rufipennis*. The numbers of *Dr. confusus* captured were too low to draw definite conclusions, and results from trapping experiments are presented in the Appendix.

6.2. Materials and Methods

Field trapping

Eight experiments evaluating nine test compounds were conducted in various locations in British Columbia (Tables 6.1., 6.2.) where the target species were prevalent. Experiments were set up in randomised complete blocks using 12-unit multiple funnel traps (Lindgren, 1983), with $\geq$ 15 m between traps. The number of replicates ranged from 9-15; some replicates were lost due to interference from weather, bears and cows. For *D. rufipennis*, replicates 1-5 for each of two experiments were set up simultaneously, and the remaining replicates were set up three weeks later. Traps baited with known attractive lures (Table 6.2.) served as positive controls, and compounds were tested in combination with the appropriate attractive lure to determine a relative increase or decrease in attraction. In each experiment, unbaited traps served as negative controls. Beetles captured in traps were stored frozen until they were identified, sexed (Lyon, 1958; Jantz & Johnsey, 1964) and counted.

Statistical analyses

All data were transformed by $\log_{10}(x+1)$ to meet the assumptions of normality and homoscedasticity and analysed by ANOVA (Proc GLM), with treatment and block as main effects, and the Ryan-Einot-Gabriel-Welsh multiple range test (Day & Quinn, 1989; SAS Institure Inc., 1990), to determine whether there were significant differences in the numbers of beetles captured among treatments. Orthogonal partitioning of treatments (Sokal & Rohlf, 1981) was performed for one experiment with catches of *D. rufipennis* to

Table 6.1. Experiment location, date, number of replicates and compounds tested for behavioural activity against three species of *Dendroctonus* in British Columbia

Target species	Trapping period	Location of field site	Exp. no.	No. of replicates	Compounds tested[a]
Coastal *D. pseudotsugae*	10 – 17 May, 2001	Malcolm Knapp Research Forest, Maple Ridge	1	13	1-octen-3-ol
Interior *D. pseudotsugae*	15 – 27 June, 2001	Alex Fraser Research Forest, 50 km east of William's Lake	2 3	15 15	1-octen-3-ol verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one), *exo*-brevicomin (*exo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane), *endo*-brevicomin (*endo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane), acetophenone, *trans*-verbenol (*trans*-4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol)

Target species	Trapping period	Location of field site	Exp. no.	No. of replicates	Compounds tested[a]
D. ponderosae	8 August – 17 September, 2001	Opax Mountain, 25 km north west of Kamloops	4	15	1-octen-3-ol
			5	15	MCOL (1-methylcyclohex-2-en-1-ol)
			6	15	MCH (3-methylcyclohex-2-en-1-one)
D. rufipennis	26 June – 18 July, 2002	Blue Jay and Augier Roads, 30 km north of Burns Lake	7	10	verbenone, *trans*-verbenol, acetophenone, 1-octen-3-ol
			8	9	nonanal, *exo*-brevicomin, *endo*-brevicomin

[a]IUPAC names if different from trivial names

Table 6.2. Chemicals used in field trapping experiments, release rates and devices

Chemical	Purity (%)	Source	Release device	Release rate (mg/24 h) and temperature[a]
ATTRACTIVE LURES				
D. pseudotsugae		Phero Tech Inc.		
ethanol	95		40 cm sleeve	40 at 20°C*
(±)-frontalin	>99		0.4 mL polyethelene vial	2.6 at 23°C*
(±)-MCOL	98		bubble cap	2.0 at 20°C*
D. ponderosae		Phero Tech Inc.		
myrcene	93		20 mL low density polyethylene bottle	95 at 23°C*
(±)-*exo*-brevicomin	99		flexlure	0.28 at 20°C*
82% (-)-*trans*-verbenol	>95		bubble cap	1.5 at 20°C*
D. rufipennis		Phero Tech Inc.		
(-)- α-pinene	>99		1.5 mL polypropylene vial	1.5 at 20°C*
(±)-frontalin	>99		0.4 mL polyethylene vial	2.6 at 23°C*
(±)-MCOL	98		bubble cap	2.0 at 20°C*
TEST COMPOUNDS				
(±)-1-octen-3-ol	98	Aldrich Chemical Co.	4 cm x 2.5 cm sealed plastic bag	5.5 at 25°C
MCH	>95	Phero Tech Inc.	bubble cap	4.0 at 20°C*

Chemical	Purity (%)	Source	Release device	Release rate (mg/24 h) and temperature[a]
(±)-MCOL	98	Phero Tech Inc.	bubble cap	2.0 at 20°C*
82% (-)-*trans*-verbenol	>95	Phero Tech Inc.	4 cm x 2.5 cm sealed plastic bag	0.9 at 25°C
75% (-)-verbenone	95	Phero Tech Inc.	4 cm x 2.5 cm sealed plastic bag	1.5 at 25°C
nonanal	95	Aldrich Chemical Co.	1.5 mL polypropylene vial with 4 pores	6.8 at 25°C
(±)-*exo*-brevicomin	99	Phero Tech Inc.	250 µL polyethylene vial with 8 pin pricks	3.1 at 25°C
(±)-*endo*-brevicomin	93	Phero Tech Inc.	250 µL polyethylene vial with 8 pin pricks	3.3 at 25°C
acetophenone	99	Aldrich Chemical Co.	1.5 mL polypropylene vial with 4 pores	6.7 at 25°C

[a]Release rates determined by Phero Tech Inc. if followed by asterisk. Otherwise, determined by DSP.

determine if the 1-octen-3-ol in combination with the attractive bait resulted in trap catches that were significantly different from those in traps with other semiochemical treatments combined. In all cases, $\alpha = 0.05$.

6.3. Results

D. pseudotsugae

In Experiment 1, there was a significant decrease in catches of both male and female coastal *D. pseudotsugae* in traps baited with 1-octen-3-ol and the attractive lure compared to traps baited with the attractive bait alone (Figure 6.1.A) (males $F = 30.02$, $df = 3,36$, $P < 0.0001$; females $F = 29.47$, $df = 3,36$, $P < 0.0001$). In the interior, 1-octen-3-ol only decreased the response of males (Figure 6.1.B) ($F = 45.06$, $df = 3,42$, $P < 0.0001$). In Experiment 3, which tested volatiles produced mainly by *D. ponderosae* against interior *D. pseudotsugae*, significantly fewer females were caught in traps with acetophenone added to the attractive lure, compared to traps with the attractive bait alone, and *trans*-verbenol significantly decreased the response of both sexes (Figure 6.1.C) (males $F = 64.4$, $df = 6,84$, $P < 0.0001$; females $F = 63.7$, $df = 6,84$, $P < 0.0001$).

D. ponderosae

1-Octen-3-ol significantly reduced the response of both sexes of *D. ponderosae* to the attractive lure (Figure 6.2.A) (males $F = 61.46$, $df = 3,39$, $P < 0.0001$; females $F = 39.06$, $df = 3,39$, $P < 0.0001$). MCOL, an aggregation pheromone of *D. pseudotsugae* and *D. rufipennis*, did not elicit a behavioural response from *D. ponderosae* (Figure 6.2.B).

Figure 6.1. Numbers of coastal (A) and interior (B) *D. pseudotsugae* captured in field trapping experiments with 1-octen-3-ol and an array of other antennally active compounds (C). Bars for each sex with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

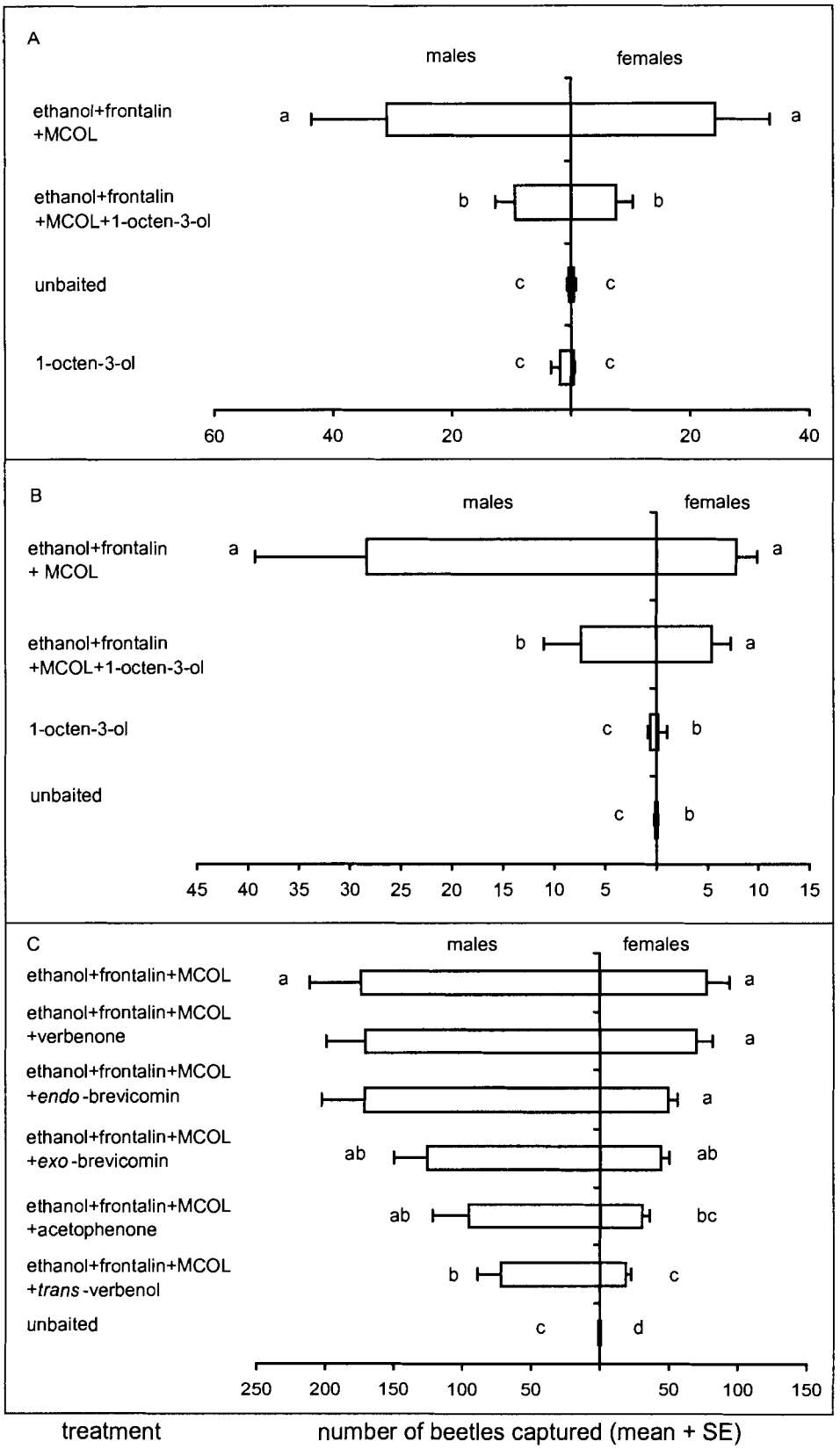
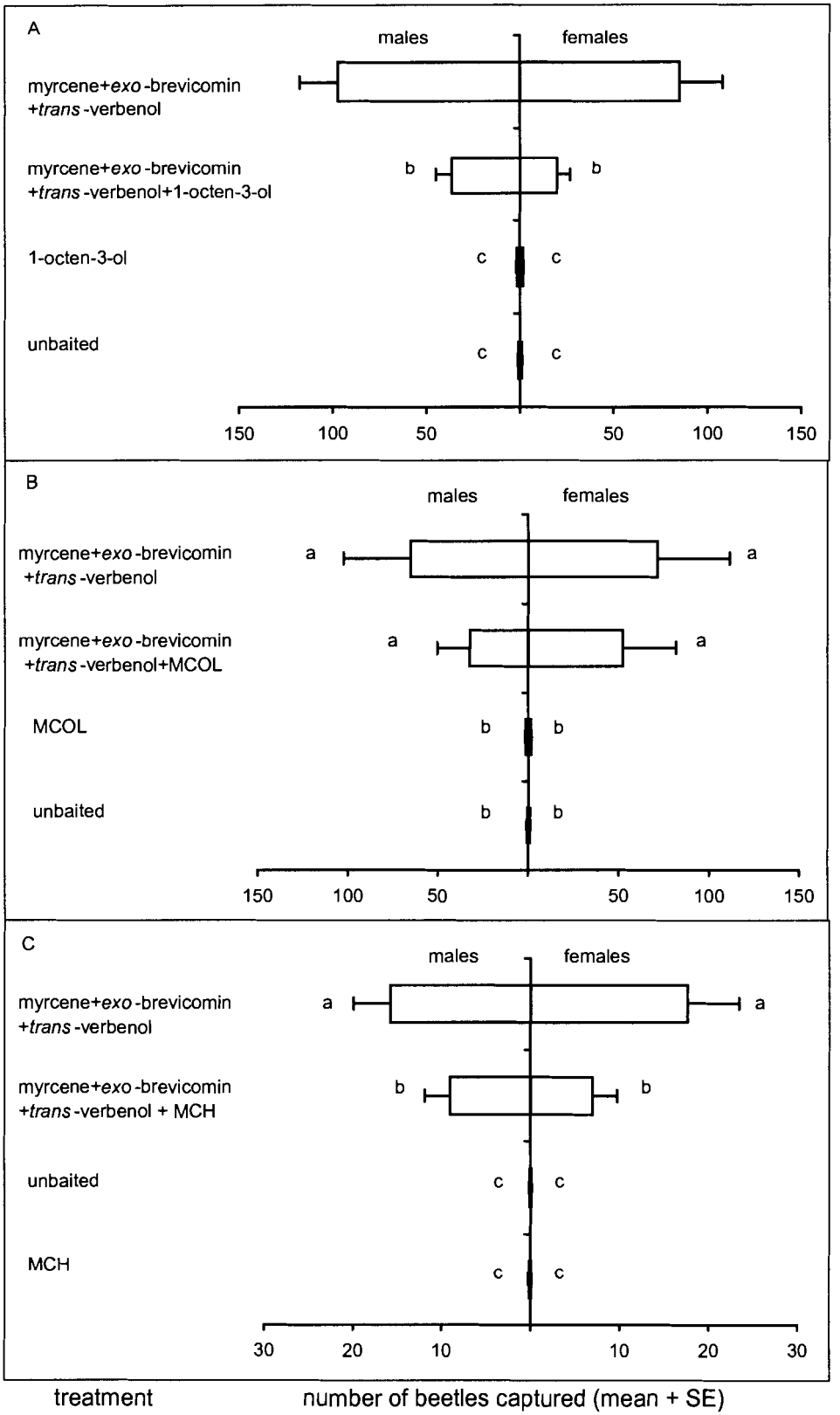

A
males
females
ethanol+frontalin +MCOL
ethanol+frontalin +MCOL+1-octen-3-ol
unbaited
1-octen-3-ol
a
a
b
b
c
c
c
c
60
40
20
20
40
B
males
females
ethanol+frontalin + MCOL
ethanol+frontalin +MCOL+1-octen-3-ol
1-octen-3-ol
unbaited
a
a
b
a
c
b
c
b
45
40
35
30
25
20
15
10
5
5
10
15
C
males
females
ethanol+frontalin+MCOL
ethanol+frontalin+MCOL +verbenone
ethanol+frontalin+MCOL +endo-brevicomin
ethanol+frontalin+MCOL +exo-brevicomin
ethanol+frontalin+MCOL +acetophenone
ethanol+frontalin+MCOL +trans-verbenol
unbaited
a
a
a
a
a
ab
ab
ab
bc
b
c
c
d
250
200
150
100
50
50
100
150
treatment
number of beetles captured (mean + SE)

Figure 6.2. Numbers of *D. ponderosae* captured in field trapping experiments with 1-octen-3-ol (A), MCOL (B) and MCH (C). Bars for each sex with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.


A
males
females
myrcene+*exo*-brevicomin
+*trans*-verbenol
myrcene+*exo*-brevicomin
+*trans*-verbenol+1-octen-3-ol
1-octen-3-ol
unbaited
b
b
c
c
c
c
150
100
50
50
100
150
B
males
females
myrcene+*exo*-brevicomin
+*trans*-verbenol
myrcene+*exo*-brevicomin
+*trans*-verbenol+MCOL
MCOL
unbaited
a
a
a
a
b
b
b
b
150
100
50
50
100
150
C
males
females
myrcene+*exo*-brevicomin
+*trans*-verbenol
myrcene+*exo*-brevicomin
+*trans*-verbenol + MCH
unbaited
MCH
a
a
b
b
c
c
c
c
30
20
10
10
20
30
treatment
number of beetles captured (mean + SE)

However, catches of both males and females were significantly reduced when MCH, the antiaggregation pheromone of *D. pseudotsugae* and *D. rufipennis* was added to the attractive aggregation pheromone lure (Figure 6.2.C) (males F = 49.97, df = 3,38, $P <$ 0.0001; females F =32.27, df = 3,38, $P < 0.0001$).

D. rufipennis

Except for 1-octen-3-ol, none of the compounds tested against *D. rufipennis* demonstrated significant behavioural activity (Figures 6.3.A,B). Although catches in traps in which 1-octen-3-ol was added to the attractive lure were not significantly different from those in traps baited with the attractive lure alone, they were reduced to a level at which they were not significantly different from those in unbaited traps (Figure 6.3.A). Moreover, orthogonal partitioning of treatments indicated that beetles responded in lower numbers to 1-octen-3-ol in combination with the attractive lure, compared to the response to all other semiochemical treatments grouped together (males: F = 8.48, df = 1,41, $P < 0.05$; females: F = 4.58, df = 1,41, $0.1 > P > 0.05$).

6.4. Discussion

1-Octen-3-ol was repellent to all three *Dendroctonus* spp. It was identified only in females of all four species. It can potentially be classified as an antiaggregation pheromone (Table 6.3.) if its biosynthesis by female beetles is elucidated, and its biological activity is determined, relative to other known antiaggregation pheromones, verbenone for *D. ponderosae* (Ryker & Yandell, 1983; Lindgren *et al.*, 1989a; Safranyik

Figure 6.3. Numbers of *D. rufipennis* captured in field trapping experiments with verbenone, *trans*-verbenol, acetophenone and 1-octen-3-ol (A) and nonanal, *exo*- and *endo*-brevicomin (B). Bars for each sex with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

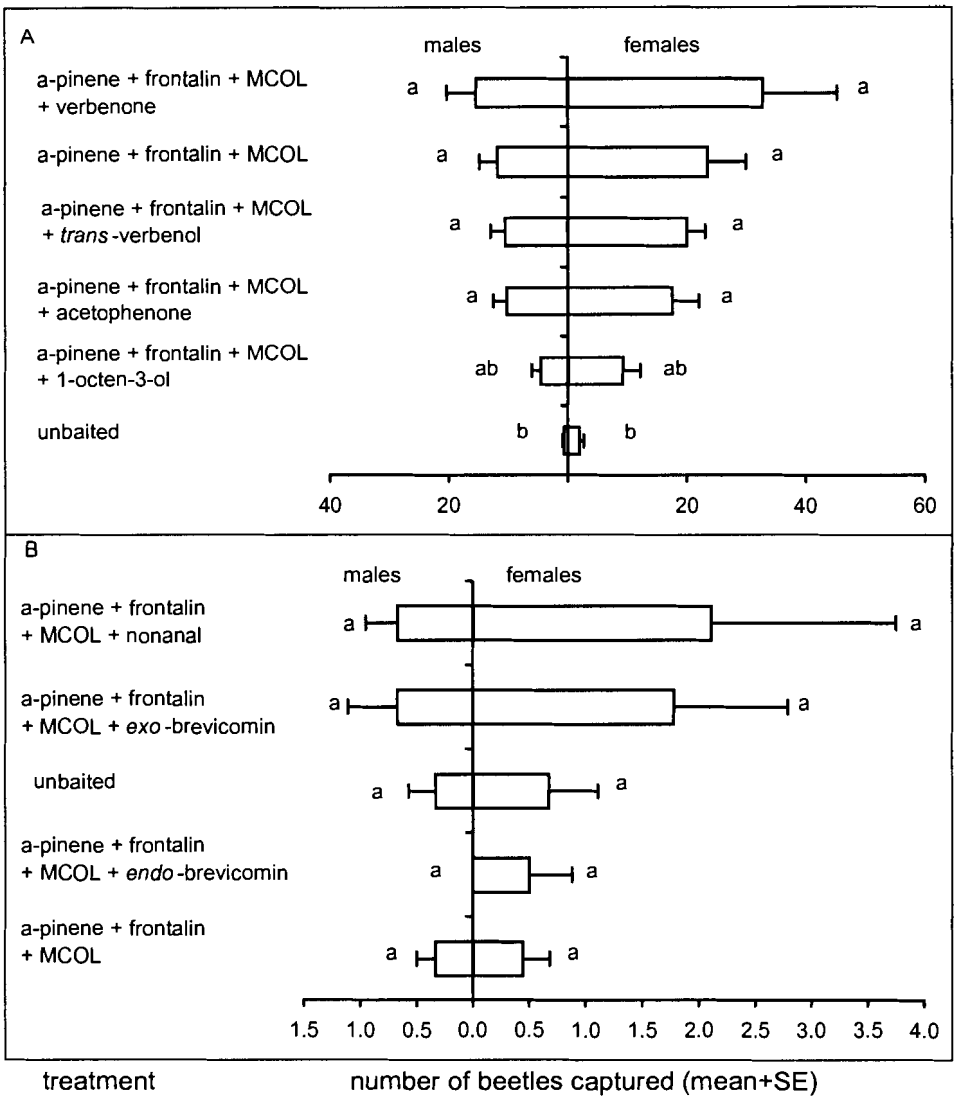

A
males
females
a-pinene + frontalin + MCOL + verbenone
a-pinene + frontalin + MCOL
a-pinene + frontalin + MCOL + trans-verbenol
a-pinene + frontalin + MCOL + acetophenone
a-pinene + frontalin + MCOL + 1-octen-3-ol
unbaited
a
a
a
a
a
a
a
a
ab
ab
b
b
40
20
20
40
60
B
males
females
a-pinene + frontalin + MCOL + nonanal
a-pinene + frontalin + MCOL + exo-brevicomin
unbaited
a-pinene + frontalin + MCOL + endo-brevicomin
a-pinene + frontalin + MCOL
a
a
a
a
a
a
a
a
a
a
1.5
1.0
0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
treatment
number of beetles captured (mean+SE)

Table 6.3. Summary of all known behaviourally active compounds in the volatiles of *D. pseudotsugae*, *D. ponderosae* and *D. rufipennis* and their verified or probable (followed by asterisk) con- and heterospecific functions.

Compound	Species and sex in which compound was detected			Verified or probable conspecific function with references	Verified or probable heterospecific function, if tested and activity shown	
	Species	Males	Females		Species	Function with references
1-octen-3-ol	*D. pseudotsugae*		+	antiaggregation pheromone (this study) *	other 2 spp.	repellent kairomone (this study) *
	D. ponderosae		+	antiaggregation pheromone (this study) *	other 2 spp.	repellent kairomone (this study) *
	D. rufipennis		+	antiaggregation pheromone (this study) *	other 2 spp.	epellent kairomone (this study) *
MCH	*D. pseudotsugae*	+	+	antiaggregation pheromone (Rudinsky, 1973; Pitman & Vité, 1974; Rudinsky *et al.*, 1974b)	*D. ponderosae*	repellent kairomone (this study)
	D. rufipennis		+	antiaggregation pheromone (Lindgren *et al.*, 1989b)		
MCOL	*D. pseudotsugae*	+	+	aggregation pheromone (Libbey *et al.*, 1983)		
	D. rufipennis	+	+	aggregation pheromone (Setter & Borden, 1999)		

Compound	Species and sex in which compound was detected			Verified or probable conspecific function with references	Verified or probable heterospecific function, if tested and activity shown	
	Species	Males	Females		Species	Function with references
trans-verbenol	*D. ponderosae*	+	+	aggregation pheromone (Pitman *et al.*, 1968; Pitman & Vité, 1969; Billings *et al.*, 1976; Libbey *et al.*, 1985)	*D. pseudotsugae*	repellent kairomone (this study)
	D. pseudotsugae	+	+	antiaggregation pheromone (this study)		
	D. rufipennis	+	+	no behavioural activity (this study)		
verbenone	*D. pseudotsugae*	+	+	no behavioural activity (this study)	*D. ponderosae*	repellent kairomone (Ryker & Yandell, 1983; Lindgren *et al.*, 1989a; Safranyik *et al.*, 1992; Chapters 3, 6)
	D. ponderosae	+	+	antiaggregation pheromone (Ryker & Yandell, 1983; Lindgren *et al.*, 1989a; Safranyik *et al.*, 1992)		
	D. rufipennis	+	+	no behavioural activity (this study)		

Compound	Species and sex in which compound was detected			Verified or probable conspecific function with references	Verified or probable heterospecific function, if tested and activity shown	
	Species	Males	Females		Species	Function with references
nonanal	*D. pseudotsugae*		+	antiaggregation pheromone* [Huber & Borden, (2001a) demontrated repellence; Chapter 3, identified in beetle volatiles]	*D. ponderosae*	repellent kairomone* (Borden *et al.*, 1998; Chapter 3)
	D. ponderosae	+	+	antiaggregation pheromone* [Borden *et al.*, (1998) demonstrated repellence]	*D. pseudotsugae*	repellent kairomone* [Huber & Borden, (2001a) demonstrated repellence]
	D. rufipennis	+	+	no behavioural activity (this study)		
exo-brevicomin	*D. ponderosae*	+		multifunctional pheromone (Borden *et al.*, 1987a)	*D. rufipennis*	repellent kairomone* (Poland & Borden 1998b)
endo-brevicomin	*D. ponderosae*	+		antiaggregation pheromone (Rudinsky *et al.* 1974a)	*D. rufipennis*	repellent kairomone* (Poland & Borden 1998a)

Compound	Species and sex in which compound was detected			Verified or probable conspecific function with references	Verified or probable heterospecific function, if tested and activity shown	
	Species	Males	Females		Species	Function with references
acetophenone	*D. ponderosae*		+	no behavioural activity (Pureswaran *et al.*, 2000)	*D. pseudotsugae*	repellent kairomone* (this study)
	D. pseudotsugae		+	antiaggregation pheromone (this study)*		
	D. rufipennis		+	no behavioural activity (this study)		
2-phenyl ethanol	*D. ponderosae*	+	+	antiaggregation pheromone (Pureswaran *et al.*, 2000)		
cis-verbenol	*D. ponderosae*	+	+	aggregation pheromone (Libbey *et al.*, 1985; Pierce *et al.*, 1987; Miller & Lafontaine, 1991)		
frontalin	*D. pseudotsugae*		+	aggregation pheromone (Pitman & Vité, 1970)	*D. ponderosae*	repellent kairomone* [Borden *et al.*, (1987a) demonstrated repellence]
	D. ponderosae	+		multifunctional pheromone (Borden *et al.*, 1987a)		
	D. rufipennis	+	+	aggregation pheromone (Kline *et al.*, 1974; Furniss *et al.*, 1976)		

Compound	Species and sex in which compound was detected			Verified or probable conspecific function with references	Verified or probable heterospecific function, if tested and activity shown	
	Species	Males	Females		Species	Function with references
verbenene	*D. rufipennis*		+	aggregation pheromone (Gries *et al.*, 1992; Borden *et al.*, 1996)		
pinocarvone	*D. ponderosae*	+	+	antiaggregation pheromone (Libbey *et al.*, 1985)		

et al., 1992) and MCH for *D. pseudotsugae* and *D. rufipennis* (Rudinsky 1973; Pitman & Vité 1974; Rudinsky *et al.* 1974b; Lindgren *et al.* 1989b). 1-Octen-3-ol has been found in one other bark beetle, specifically in the head space volatiles of the European species *X. bispinus*, to which it was repellent (Klimetzek *et al.*, 1989). Although it occurs in nature as the *R* -(–) enantiomer (Pierce *et al.*, 1989; McMahon *et al.*, 2001), both the *R* -(–) enantiomer and the racemate were attractive to bont ticks (McMahon *et al.*, 2001), as well as to cucujid beetles *O. surinamensis* and *O. mercator* (Pierce *et al.*, 1989), for which it is an aggregation pheromone. 1-Octen-3-ol was also identified in the bark volatiles of European birch and aspen (Zhang *et al.*, 2000). The commercially available racemate was repellent in this study at the release rate tested, and although the separate enantiomers were not tested, it is likely that the *R*-(–)-enantiomer, identified in volatiles of female bark beetles (Chapter 3), would have the same repellent effect. 1-Octen-3-ol is derived from the oxidation of linoleic acid (Tressl *et al.*, 1982) and has been identified in the volatiles of fungi (Kaminski *et al.*, 1972). Thus for bark beetles, it could serve as a kairomonal indicator of fungal activity in hosts that are no longer suitable for colonisation due to an advanced stage of beetle attack and the onset of fungal deterioration. All three *Dendroctonus* species are sympatric and have partially overlapping seasonal flight periods. 1-Octen-3-ol, if produced by beetles, could indicate unacceptable hosts or nonhosts and serve as a kairomone, being beneficial to the species in flight during host selection, with no apparent interspecific benefit to the emitter. The results also suggest that 1-octen-3-ol could be used in combination with other repellent materials to protect trees from attack by any of the three species tested.

trans-Verbenol, an aggregation pheromone produced by female *D. ponderosae* (Pitman *et al.*, 1968; Pitman & Vité, 1969; Billings *et al.*, 1976; Libbey *et al.*, 1985) was repellent to both sexes of *D. pseudotsugae* at the dose tested and can function as a kairomone for *D. pseudotsugae.* It occurs in the volatiles of both sexes of *D. pseudotsugae* (Rudinsky *et al.*, 1972; Chapter 3) and could also potentially function as an antiaggregation pheromone. In laboratory bioassays, *trans*-verbenol was repellent to *D. pseudotsugae* at high concentrations, weakly repellent in lower concentrations, but had no effect in field tests either alone or in combination with host volatiles (Rudinsky *et al.*, 1972). Although it elicited antennal response, *trans*-verbenol had no behavioural effect on *D. rufipennis* in this study.

Verbenone is found in the volatiles of several *Dendroctonus* spp. and commonly disrupts aggregation in species such as the southern pine beetle, *D. frontalis*, the western pine beetle, *D. brevicomis* (Renwick & Vité, 1970), the roundheaded pine beetle, *D. adjunctus* (Livingston *et al.*, 1983) and *D. ponderosae* (Ryker & Yandell, 1983; Lindgren *et al.*, 1989a; Safranyik *et al.*, 1992). It was identified in the volatiles of *D. pseudotsugae* and *D. rufipennis* (Chapter 3), but had no behavioural effect on either species at the dose tested (Figures 6.1C, 6.3A).

exo-Brevicomin is produced by *D. ponderosae* (Rudinsky *et al.*,1974a; Pureswaran *et al.* 2000), and is a multifunctional pheromone, being aggregative at low doses, and antiaggregative at high doses (Borden *et al.*, 1987a). *endo*-Brevicomin was found to be repellent to *D. ponderosae* (Rudinsky *et al.*, 1974a). Poland & Borden (1998a) found that (±)- or (+)-*exo*- and (±)- or (+)-*endo*-brevicomin significantly reduced

trap catches of *D. rufipennis,* but racemates of both compounds at the release rates tested had no effect on *D. pseudotsugae* or *D. rufipennis* in this study. Therefore their role as semiochemicals for these species is uncertain (Table 6.3.).

Nonanal, detected in the volatiles of all three species (Chapter 3) also occurs in the volatiles of lodgepole pine (Pureswaran *et al.* 2000), and in the bark of angiosperms (Huber *et al.*, 2000). In combination with alcohols and aldehydes identified from angiosperms, nonanal significantly reduced the catches of *D. ponderosae* (Borden *et al.*, 1998) and *D. pseudotsugae* (Huber & Borden, 2001a) to attractant baited traps. When tested against *D. rufipennis*, it had no effect, but this could be due to the low number of beetles captured in this experiment (Figure 6.3.B).

Acetophenone occurred in the volatiles of females in all three species (Chapter 3). It was previously found in crushed abdomens of female *D. ponderosae* (Pierce *et al.*, 1987) and in the emitted volatiles of both sexes (Pureswaran *et al.*, 2000). Although it had no behavioural activity in *D. ponderosae* (Pureswaran *et al.* 2000) or *D. rufipennis* (Figure 6.3.A), at the dose tested, it significantly reduced the response of female *D. pseudotsugae* to the attractive bait (Figure 6.1.C), suggesting that it could serve as a repellent kairomone during host selection (Table 6.3.).

1-Methylcyclohex-2-en-1-ol (MCOL), an aggregation pheromone of *D. pseudotsugae* (Libbey *et al.*, 1983) and *D. rufipennis* (Borden *et al.*, 1996; Setter & Borden, 1999) had no significant effect on the behavioural activity of *D. ponderosae.* However, MCH an antiaggregation pheromone of *D. pseudotsugae* (Rudinsky, 1973; Pitman & Vité, 1974; Rudinsky *et al.*, 1974b) and *D. rufipennis*

(Lindgren *et al.*, 1989b), inhibited the response of both sexes of *D. ponderosae* to the aggregation pheromone bait, indicating that it can function as a repellent kairomone (Table 6.3.). Although it was found in relatively low amounts in the volatiles of *D. ponderosae* (Rudinsky *et al.*, 1974a), it could potentially also serve as a minor antiaggregation pheromone. MCH is registered as a pesticide against *D. pseudotsugae* in the USA, and may have potential as a supplement to verbenone in protecting trees from attack by *D. ponderosae*.

A summary of all known behaviourally active semiochemicals produced by all three *Dendroctonus* spp. reveals a complex of compounds that are often shared between species and have variable pheromonal and kairomonal activity (Table 6.3.). Host selection in these species would be driven partly by attraction to pheromones from conspecifics alone or in combination with attractive host kairomones (Chapters 2, 5), and partly by avoidance of kairomones emitted by heterospecific beetles in nonhost conifers (Chapter 6), repellent kairomones from nonhost angiosperms (Huber & Borden, 2001a), and possibly repellent kairomones from nonhost conifers (Chapter 5). Although, there is only partial overlap in the dispersal and aggregation phase of species in my study, attraction to nonhosts may occur by kairomonal cross attraction to pheromones of heterospecific beetles (because species have aggregation pheromones in common) in that host, and can be deterred or the resource partitioned by mutually repellent synomones from heterospecific beetles (Borden, 1997).

Inhibition of attraction to semiochemicals produced by heterospecifics is occurs among coniferophagous Scolytidae. Although there may be only partial temporal overlap

in dispersal and aggregation phases in my study species, perception and avoidance of compounds emitted by heterospecifics attacking nonhosts can help in imparting greater host specificity during dispersal. *Dendroctonus pseudotsugae* and *D. ponderosae* initiated attack on nonhosts when they were baited with their aggregation pheromones (Chapter 2). I have captured *D. pseudotsugae,* which typically fly in early summer, in my traps during the flight period of *D. ponderosae* much later in the season. The flight periods of *D. pseudotsugae* and *D. rufipennis* also overlap spatially and temporally. Considerable redundancy in attractive and repellent semiochemical signals exists in bark beetles (Borden *et al.*, 1990). Heterospecific kairomones emitted by beetles infesting nonhost conifers can therefore reinforce nonhost cues and serve as a factor that mediates host selection.

7. The myrcene enigma: Why is myrcene the most effective synergist for the mountain pine beetle's aggregation pheromones?

7.1. Introduction

Following the discovery of female-produced *trans*-verbenol (Pitman *et al.*, 1968; Pitman, 1971) and male-produced *exo*-brevicomin (Rudinsky *et al.*, 1974a) as major aggregation pheromones of *D. ponderosae*, the monoterpene myrcene was identified as the most efficient synergist for these pheromones in traps (Conn *et al.*, 1983; Borden *et al.*, 1987a) and on trees (Borden *et al.*, 1983). However, myrcene constitutes < 5% of the volatiles of most North American pines infested by *D. ponderosae* (Smith, 2000), and only 2.6% and 1.1% of the monoterpene content of the bole and foliage volatiles, respectively, of lodgepole pine, *P. contorta* var. *latifolia*, the beetle's major host in interior B.C. (Chapter 4). In Chapter 5, I found myrcene to be a more effective pheromone synergist than simplified synthetic blends of monoterpenes that each constituted ≥ 5% of bole or foliage volatiles of lodgepole pine.

I hypothesised that the lack of pronounced synergism by this simple lodgepole pine blend could be attributed to its incomplete nature, or that the preferred host of *D. ponderosae* is some other pine, e.g. *P. ponderosa* (Smith, 2000), that may contain more myrcene than lodgepole pine in its oleoresin. In this chapter, I tested simple and complete synthetic blends of lodgepole pine volatiles in comparison with myrcene in

pheromone-baited traps, compared the monoterpene composition of bole and foliage volatiles of ponderosa pine to that of lodgepole pine, and examined the literature to determine if myrcene is a major component of any other *Pinus* spp. in North America.

7.2. Materials and Methods

Two separate experiments evaluated the synergistic effect of myrcene or simple and complete synthetic blends of bole or foliage volatiles respectively, of lodgepole pine from 2 to 22 August, 2002 on Opax Mountain, 25 km northwest of Kamloops, B.C. Ten replicates of 12-unit multiple funnel traps (Lindgren, 1983), were set up in randomised complete blocks, with $\geq$ 15 m between traps. Treatments were: 1) aggregation pheromone bait (positive control), 2) unbaited traps (negative control), 3) bait plus myrcene, and 4) bait plus simple lodgepole pine blend (bole and foliage in separate experiments) (Table 7.1) and 5) bait plus complete lodgepole pine blend (bole and foliage in separate experiments) (Table 7.1). Myrcene and the synthetic blends were released from low density polyethylene bottles at 95 mg / 24 h at 23°C (myrcene) and 140 mg / 24 h at 25°C (synthetic blends) as determined in the laboratory. (±)-*exo*-Brevicomin, 99% pure, and 82% (-)-*trans*-verbenol, > 95% pure, were respectively released at 0.28 mg / 24h at 20 °C from polyurethane flexlures and 1.5 mg / 24 h from bubble caps (Phero Tech Inc., Delta, B.C.). Captured beetles were frozen until they were identified, sexed (Lyon, 1958) and counted. All data were transformed by $\log_{10}(x+1)$ to meet the assumptions of normality and homoscedasticity, and analysed by ANOVA (Proc GLM) (SAS Institute Inc. 1990) with treatment and block as main effects, and the Ryan-Einot-Gabriel-Welsh multiple

Table 7.1. Composition and release rates of synthetic blends of lodgepole pine volatiles.

Compound	Percent composition		Percent purity	Source
	bole	foliage		
SIMPLE BLENDS				
(-)-α-pinene	5.94	8.4	98	Aldrich Chemical Co.
(-)-β-pinene	19.15	51.3	99	Aldrich Chemical Co.
(+)-3-carene	6.98		90	Aldrich Chemical Co.
(-)-β-phellandrene	60.73	40.3	60-65	H.D. Pierce, Jr., Simon Fraser University
(-)-limonene	7.19		96	Aldrich Chemical Co.
COMPLETE BLENDS				
(-)-α-pinene	4.9	7.7	98	Aldrich Chemical Co.
(+)-α-pinene	1.91	1.2	98	Aldrich Chemical Co.
(-)-camphene	2.53	0.2	80	Aldrich Chemical Co.
(+)-camphene	1.42		80	Aldrich Chemical Co.
(-)-sabinene	0.6	0.1	19-23	H.D. Pierce, Jr.
(+)-sabinene	0.57	0.2	99	Indofine Chemical Co. Inc.
(-)-β-pinene	15.8	47.0	99	Aldrich Chemical Co.
(+)-β-pinene	1.77	1.1	98	Aldrich Chemical Co.
myrcene	2.6	1.1	90	Aldrich Chemical Co.
(+)-3-carene	5.76	2.3	90	Aldrich Chemical Co.
α-terpinene	0.26		89	Sigma Chemical Co.
p-cymene	1.61		99	Aldrich Chemical Co.
(-)-β-phellandrene	50.1	36.9	60-65	H.D. Pierce, Jr.
(-)-limonene	5.93	0.7	96	Aldrich Chemical Co.
(+)-limonene	2.24	0.5	97	Aldrich Chemical Co.
γ-terpinene	0.16		95	Aldrich Chemical Co.
terpinolene	0.99	0.2	90	Fluka Chemical Co.
(-)-bornyl acetate	0.85	0.8	97	Sigma Chemical Co.

range test (Day & Quinn, 1989), to determine if there were differences in the numbers of beetles captured among treatments. In all cases, $\alpha = 0.05$.

Samples of bole and foliage from 10 ponderosa pines $\geq$ 25 cm in diameter at a height of 1.3 m, and at least 500 m apart were collected on 14 August, 2002, on Whipsaw Road, 14 km south of Princeton, B.C. A sample of bole tissue from each tree including outer bark, phloem, and sapwood was removed using a sharp hatchet and placed in a glass jar. From the same tree, a branch of foliage at a height of about 3.5 m was clipped using a pole-pruner, and placed in a plastic bag. Samples were stored over dry ice for transport to the laboratory, and at -15°C in a freezer until analysed. Monoterpenes from homogenised bole and foliage were extracted in hexane and then analysed by gas-chromatography (GC) and GC-mass spectrometry, with n-heptane as the internal standard, using the procedure described by Tomlin *et al.* (1997).

7.3. Results and Discussion

Traps baited with myrcene, in combination with the aggregation pheromone bait caught at least seven times more *D. ponderosae* than traps with either simple or complete blends of lodgepole pine bole or foliage volatiles (Figure 7.1). There was no difference in the synergistic effect between simple and complete blends, except for a higher response of males to the complete blend than the simple blend of bole volatiles.

Bole and foliage volatiles of ponderosa pine in an area infested by *D. ponderosae* contained < 3 % myrcene (Table 7.2), a content similar (2.6% and 1.1% of the monoterpene content of the bole and foliage volatiles, respectively), to that from

Figure 7.1. Numbers of *D. ponderosae* captured in field trapping experiments testing a pheromone bait in combination with myrcene or reconstituted blends of lodgepole pine bole or foliage volatiles. Treatments are ordered for female (the first attacking sex) trap catch. Bars for each sex and experiment with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

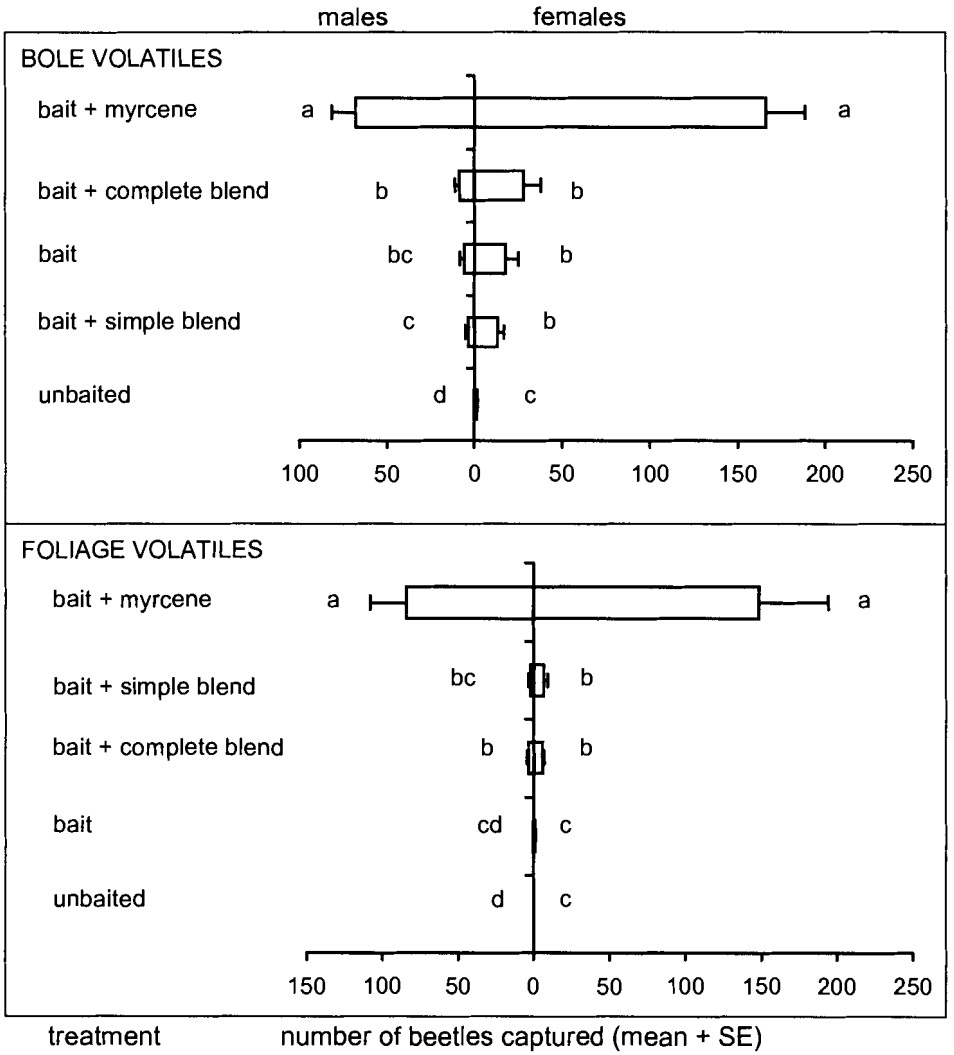

males
females
BOLE VOLATILES
bait + myrcene
bait + complete blend
bait
bait + simple blend
unbaited
a
a
b
b
bc
b
c
b
d
c
100 50 0 50 100 150 200 250
FOLIAGE VOLATILES
bait + myrcene
bait + simple blend
bait + complete blend
bait
unbaited
a
a
bc
b
b
b
cd
c
d
c
150 100 50 0 50 100 150 200 250
treatment
number of beetles captured (mean + SE)

Table 7.2. Monoterpene composition of bole and foliage volatiles of ponderosa pine sampled near Princeton, B.C. Mean amounts in micrograms per gram dry weight of tissue.

	Bole		Foliage	
Monoterpene	Mean ± SE	%	Mean ± SE	%
(-)-α-pinene	3.22 ± 0.57	5.81	27.73 ± 3.39	12.84
(+)-α-pinene	10.25 ± 1.63	18.49	12.3 ± 2.58	5.7
(-)-camphene	0.25 ± 0.04	0.45	0.83 ± 0.17	0.38
(+)-camphene	0.15 ± 0.03	0.27	0.38 ± 0.08	0.17
(+)-sabinene	0.43 ± 0.23	0.7	0	
(-)-β-pinene	3.2 ± 0.41	5.77	147.95 ± 19.74	68.53
myrcene	1.25 ± 0.26	2.25	1.85 ± 0.42	0.86
(+)-3-carene	25.47 ± 4.7	45.95	19.95 ± 1.59	9.24
α-terpinene	0.18 ± 0.09	0.32	0.12 ± 0.01	0.06
p-cymene	0.31 ± 0.04	0.56	0.16 ± 0.03	0.07
(-)-β-phellandrene	0.86 ± 0.26	1.55	1.74 ± 0.27	0.81
(+)-limonene	5.3 ± 1.0	9.56	1.49 ± 0.17	0.69
γ-terpinene	0.3 ± 0.04	0.54	0.26 ± 0.02	0.12
terpinolene	3.88 ± 1.03	6.99	0.98 ± 0.08	0.45
(-)-bornyl acetate	0.38 ± 0.14	0.69	0.16 ± 0.04	0.07

lodgepole pine from the same location (Chapter 4). This is consistent with Mirov's (1961) and Zavarin & Cobb's (1970) studies of ponderosa pine xylem oleoresin in the Sierra Nevada, but is less than the percentage obtained by Smith (1964) (9.2-17.5 %) in the same region, and 8.12 % in xylem oleoresin, and 2.54 % in needles from Colorado (Latta *et al.*, 2000). In no case was myrcene the predominant monoterpene.

In their study on the phylogeny of host use, Kelley & Farrell (1998) concluded that the genus *Pinus* is the host of the most recent common ancestor of *Dendroctonus* spp. The attraction of *D. ponderosae* to myrcene, a relatively minor monoterpene of most North American pines (Smith, 2000) is intriguing, because unlike other species of *Dendroctonus*, *D. ponderosae* demonstrates only weak (Gara *et al.* 1984; Moeck & Simmons, 1991) or no primary attraction to host volatiles (Hynum & Berryman, 1980; Moeck *et al.*, 1981; Chapter 5). One explanation for this phenomenon is that *D. ponderosae* could have evolved to respond positively to high levels of myrcene in induced (traumatic) resin. The amount of traumatic resin increased 4.5 times in reaction tissue of lodgepole pine, seven days after inoculation of fungi vectored by *D. ponderosae* (Raffa & Berryman, 1983). However, relative amounts of individual monoterpenes, including myrcene, remained unchanged in wounded lodgepole pine tissue (7.9 % myrcene in wounded tissue *versus* 8.5% in uninjured tissue) (Shrimpton, 1973), similar to the findings for red pine, *Pinus resinosa* Ait. and jack pine, *Pinus banksiana* Lamb (Raffa & Smalley, 1995).

Another possible explanation is that *D. ponderosae* retains an attractive response to myrcene from a more ancient pine host. For example, Smith (2000) found that 23

whitebark pines, *Pinus albicaulis* Engelm. sampled in the Paulina Mountains in Oregon had a mean content of 27.8 % myrcene in their xylem oleoresin; one tree contained 68.8 %, the highest amount of myrcene documented in any pine. A further 62 trees from Mt. Shasta, California contained an average of 28.1 % myrcene. The third most prevalent monoterpene in *P. albicaulis* was terpinolene (Smith, 2000), a compound ranked second in bioactivity to myrcene in field experiments (Borden *et al.*, 1983; Conn *et al.*, 1983). Fossil evidence suggests that limber pine, *Pinus flexilis* James, whose pollen is difficult to distinguish from *P. albicaulis* (R.W. Mathewes, pers. comm.) grew as much 1100m below its modern elevational level in the Great Basin during the late Pleistocene (Thompson, 1990). This indicates that *P. albicaulis* could also have grown in lower elevations in the Pleistocene.

Although *P. albicaulis* is a suitable host for *D. ponderosae*, its current distribution, in isolated, exlcusively high elevation habitats, is too severe for beetle populations to thrive (Logan & Powell, 2001). As *D. ponderosae* expanded its host range to exploit other species such as lodgepole pine, the most widely distributed pine in North America (Critchfield & Little, 1966), it may have adapted to an alternative host selection strategy characterised by a lack of long-range olfactory discrimination between host and nonhost conifers (Chapter 2, 5), random landing on both host and nonhost trees (Hynum & Berryman, 1980), and subsequent release of potent aggregation pheromones (Pitman *et al.*, 1968; Pitman, 1971; Borden, 1974; Rudinsky *et al.*, 1974a; D.L. Wood, 1982), that concentrated mass attack on acceptable hosts.

8. Conclusions

From my study on the role of kairomones and pheromones in the host selection of *D. pseudotsugae*, *D. ponderosae*, *D. rufipennis* and *Dr. confusus*, the following conclusions can be drawn.

1. All four species oriented toward and landed on nonhost conifers baited with their aggregation pheromones indicating that there was no strong long-range repellence caused by nonhost volatiles.

2. Neither *D. rufipennis* nor *Dr. confusus* attempted to establish galleries on nonhosts. Few attacks were initiated by *D. pseudotsugae* and *D. ponderosae* on nonhosts lodgepole pine and Douglas-fir, respectively. Most attacks did not reach the phloem tissue, and in no case were they numerous enough to have produced a significant source of aggregation pheromone. Therefore, employing pheromone baited nonhost trap trees would not be an effective management tactic.

3. Lack of strong repellence from nonhost conifers partly supports the hypothesis of random landing followed by close-range olfactory or gustatory rejection of nonhosts by tree-killing bark beetles.

4. A comparative study of electrophysiological responses of four species of tree-killing bark beetles to volatiles from a) bole and foliage of host and nonhost conifers, and b) con- and heterospecific beetles by GC-EAD, revealed 18 monoterpenes in conifers and nine compounds in the volatiles of beetles that elicited antennal responses. These compounds were thus identified as candidate semiochemicals with potential behavioural roles in host location and discrimination.

5. There was no qualitative difference in the monoterpene constitution of the four species of conifers and very little difference across beetle species in their antennal response to host and nonhost compounds, suggesting that beetles would need to detect differences in the ratios of different monoterpenes in conifers to discriminate among them.

6. Extractions of bole and foliage tissues of the four species of conifers in three locations in B.C., followed by MANOVA and principal component analyses on monoterpene amounts, revealed that although the four species were qualitatively similar, significant interspecific differences existed in their quantitative monoterpene profiles. These differences were large enough to justify testing the hypothesis that host selection by coniferophagous bark beetles may depend in part on perception of, and behavioural response to quantitatively distinct blends of monoterpenes.

7. There was significant variation in the monoterpene composition between bole and foliage volatiles in all four conifer species. The results of my foliage profiles support Hart's (1987) cladistic relationship of Pinaceae based on classical taxonomy, in which *Pinus* and *Picea* share a more recent common ancestor than the clade giving rise to *Pseudotsuga* and *Abies*, but the results of my bole profiles do not, demonstrating the risk of drawing chemosystematic conlusions based on limited sampling.

8. Principal components analysis revealed that the monoterpene profiles of bole and foliage volatiles, particularly the latter, of coastal Douglas-fir were different from those of interior trees, supporting von Rudloff's (1972a) hypothesis that coastal and interior populations represent distinct chemotypes. A lack of complete separation of bole volatiles between trees from Maple Ridge and Princeton suggests that the Maple Ridge population is more closely related to trees from Princeton than to trees in the more northern interior locations.

9. In field experiments, major bole and foliage volatiles of Douglas-fir, increased the attraction of coastal and interior *D. pseudotsugae* to pheromone-baited traps. Primary attraction to bole volatiles was observed in interior *D. pseudotsugae*. Beetles were significantly less attracted to the pheromone bait when it was combined with volatiles of lodgepole pine, or interior fir. Thus both primary attraction, and the capacity for long-range discrimination between hosts and nonhosts are demonstrated for this species.

10. The monoterpene myrcene synergised attraction of *D. ponderosae* to aggregation pheromones, but there was no evidence of primary attraction to host volatiles or discrimination among volatiles from the four species of conifers. Therefore, the hypothesis of random landing on hosts and nonhosts and close-range discrimination between them (in the absence of aggregation pheromones) is supported for *D. ponderosae*.

11. There was significant attraction of the spruce beetle, *D. rufipennis*, to bole and foliage volatiles of interior spruce, supporting the hypothesis of primary attraction, but beetles did not discriminate among volatiles of four sympatric conifers when they were combined with pheromone baits.

12. 1-Octen-3-ol, found in the volatiles of females of all four species of bark beetles, decreased the response of male and female coastal *D. pseudotsugae,* male interior *D. pseudotsugae,* both sexes of *D. ponderosae* and male *D. rufipennis* to their aggregation pheromones, suggesting its probable dual function as a new antiaggregation pheromone and repellent kairomone for bark beetles.

13. Acetophenone, identified in the volatiles of females of all four species of bark beetles, decreased the response of female interior *D. pseudotsugae* to traps baited with aggregation pheromones. *trans*-Verbenol, a potent aggregation pheromone of *D. ponderosae*, also decreased the response of both sexes of interior *D. pseudotsugae.*

The former compound thus has a probable dual role as an antiaggregation pheromone and a repellent kairomone for *D. pseudotsugae*, while the latter can be tentatively classified as a repellent kairomone.

14. 3-Methyl-2-cyclohexen-1-one (MCH), an antiaggregation pheromone of *D. pseudotsugae* and *D. rufipennis,* decreased the response of both sexes of *D. ponderosae* to traps baited with aggregation pheromones, suggesting its function as a repellent kairomone for *D. ponderosae.*

15. The results underlying conclusions 12-14 support a general hypothesis that tree-killing bark beetles can perceive signals emitted by heterospecifics that attack nonhosts, and potentially use them alone or in combination with nonhost volatiles to avoid attacking the wrong species of conifer.

16. Although the monoterpene myrcene constitutes < 5% of the volatiles of lodgepole pine and ponderosa pine, both common hosts of *D. ponderosae,* it was a much more effective synergist to aggregation pheromones of *D. ponderosae* than partial or complete synthetic blends of lodgepole pine bole or foliage volatiles. To explain this enigma, I propose the hypothesis that attraction to myrcene may be an evolutionary vestige of attraction to a more ancient pine host, possibly whitebark pine, *P. albicaulis* that is rich in myrcene. As *D. ponderosae* expanded its host range to exploit many other species of pines, with variable monoterpene profiles, it may have

lost the capacity to discriminate among host and nonhost volatiles and evolved a new strategy based on random landing on hosts and nonhosts, supplemented by increased reliance on potent aggregation pheromones released by beetles on acceptable hosts.

9. Appendix

Results of field trapping experiments with *Dr. confusus*

Dr. confusus was trapped on Goat Mountain, 45 km south of Lumby, from 27 July to 16 September, 2002. For positive controls, 99 % pure (±)-*exo*-Brevicomin (obtained from Phero Tech Inc., Delta, B.C.) was released from 1.5 mL polypropylene vials at 1.7mg / 24 h, at 23°C.

Table 9.1. Numbers of *Dr. confusus* captured in field trapping experiments with (1) bole and foliage volatiles of interior fir (IF), (2) bole and (3) foliage volatiles from four species of conifers, and (4) volatiles from beetles. Acronyms for source populations of trees given in Table 5.2. Means for each sex and experiment with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

		No. of beetles captured (mean + SE)	
Experiment	Treatment	males	females
1. Bole and	Unbaited	0.09 + 0.09 a	0.09 + 0.09 a
foliage	bole volatiles	0.36 + 0.28 a	0.18 + 0.18 a
volatiles of	foliage volatiles	0.27 + 0.14 a	0.55 + 0.21 a
interior fir	bole + foliage volatiles	0.27 + 0.19 a	0.36 + 0.20 a
(N = 11)			
2. Bole	bait + IDF	2.50 + 0.42 a	2.17 + 0.47 a
volatiles of	bait	1.17 + 0.39 b	1.17 + 0.52 ab
four species of	unbaited	0.17 + 0.11 b	0.25 + 0.18 b

Experiment	Treatment	No. of beetles captured (mean + SE) males	females
conifers	bait + ISP	1.33 + 0.54 b	1.33 + 0.61 ab
(N = 12)	bait + IF	0.67 + 0.26 b	0.75 + 0.28 ab
	bait +LP	0.58 + 0.25 b	0.58 + 0.23 b
3. Foliage	bait + IDF	2.27 + 0.71 a	0.72 + 0.41 a
volatiles of	bait + ISP	1.73 + 1.00 ab	0.73 + 0.24 a
four species of	bait + IF	1.36 + 0.61 ab	0.36 + 0.15 a
conifers	bait	1.73 + 0.49 ab	1.00 + 0.33 a
(N = 11)	bait + LP	0.64 + 0.24 ab	0.18 + 0.12 a
	unbaited	0.00 + 0.00 b	0.18 + 0.12 a
4. Volatiles	bait + *trans*-verbenol	0.69 + 0.33 a	0.23 + 0.12 a
from beetles	bait + acetophenone	0.41 + 0.19 a	0.17 + 0.11 a
(N = 13)	bait + verbenone	0.33 + 0.18 a	0.08 + 0.08 a
	bait + nonanal	0.38 + 0.18 a	0.31 + 0.17 a
	bait + 1-octen-3-ol	0.23 + 0.12 a	0.15 + 0.10 a
	bait	0.0 + 0.00 a	0.08 + 0.08 a
	unbaited	0.00 + 0.00 a	0.00 + 0.00 a

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