POTENTIAL ADJUVANTS TO ATTRACTIVE BAITS FOR THE SPRUCE BEETLE, 

*Dendroctonus rufipennis* Kirby (COLEOPTERA:SCOLYTIDAE) 

by 

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Potential Adjuvants to Attractive Baits for the Spruce Beetle,

Dendroctonus rufipennis Kirby (Coleoptera: Scolytidae).

Author:

(signature)

Robert R. Setter

(name)

AUG. 15/97

(date)
DEDICATION

This work is deserving of two dedications. First to Drs. Mark Winston, Gerhard Gries, and John Borden, for providing the inspiration to study entomology, and subsequently to my wife Debbi, my mother and my father, for their patience and encouragement in the pursuit of my studies.
ABSTRACT

The ability of several compounds to enhance the attraction of spruce beetles to the aggregation pheromone frontalin was tested in British Columbia in randomized complete block experiments using multiple-funnel traps or baited trees. Frontalin was only moderately attractive alone in traps in northcentral B.C. MCOL (1-methyl-2-cyclohexen-1-ol) of any enantiomeric composition significantly enhanced the attraction of both sexes to traps baited with frontalin. The lack of an enantiospecific preference for MCOL supports the hypothesis of pheromone-based spruce beetle “ecotypes”, aligns beetles in northcentral B.C. with those in southwestern B.C. and northern Alberta, and indicates that they are distinct from populations in southern B.C. and Alaska. MCOL was proportionally more attractive than frontalin to males early in the season, indicating that its function may be biased towards that of a sex pheromone, while the function of frontalin may be biased towards aggregation. Neither MCOL nor seudenol (3-methyl-2-cyclohexen-1-ol) enhanced the efficacy of frontalin as a tree bait in northern B.C., and frontalin was highly effective as a tree bait alone, without the host kairomone α-pinene. Coupled gas chromatographic-electroantennographic analysis of the frass volatiles from female spruce beetles revealed two potential new pheromones: ethyl crotonate and 3,3-MCH (3-methyl-3-cyclohexen-1-one). Trapping experiments in southcentral B.C. tested these two compounds, as well as the hypothesized pheromone 3,3-MCH-ol, for ability to enhance captures of spruce beetles in frontalin baited traps. Both ethyl crotonate and 3,3-MCH-ol caused a significant enhancement in one of two experiments, suggesting that they are new pheromones for the spruce beetle. In baited tree experiments in southcentral B.C. neither ethyl crotonate, 3,3-MCH-ol, nor MCOL caused a significant increased in attack density over that induced by frontalin, but a blend of all three test compounds together did so, providing some support for the pheromone hypothesis. My results suggest that MCOL, ethyl crotonate or 3,3-MCH-ol could be used to enhance the efficacy of monitoring traps for the spruce beetle. However, the power of frontalin alone at a release rate as low as 0.1 mg per 24 h, indicates that there is no compelling argument for changing the currently-used operational tree baits, other than to lower the dose of frontalin and eliminate α-pinene.
ACKNOWLEDGEMENTS

For advice, guidance and assistance in the completion of this thesis I particularly wish to thank Dr. J.H. Borden. I also wish to thank Dr. G. Gries, Dr. B.S. Lindgren, Dr. D.R. Miller, Leslie Chong, Therese Poland, Jorge Macias - Samano, Ian Wilson, Jerry Carlson, Patrick Koehn, Michael Wells, Debbi Seymour, Darrell Devlin, Pat Byrne, Margo Hollinger, Glen Esdale, J.P. Lafontaine, Dr. H. Pierce, participating members from the lab of Dr. C. Oehlschlager in the Department of Chemistry at S.F.U., and participating employees from Bugbusters Pest Management Inc., Fletcher Challenge Canada and Finlay Forest Industries Mackenzie Woodlands Divisions, Weyerhauser Canada's Princeton and Okanagan Falls Divisions, the B.C. Ministry of Forests Mackenzie Forest District, and Phero Tech Inc.

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INTRODUCTION

Over the past decade semiochemical-based technology has made a substantial contribution to the field of integrated pest management. Technological advances in the identification and controlled release of synthetic compounds, have led researchers into deep exploration of the complex chemical language that insects use (Borden 1995). As the understanding of this language continues to increase, so does the ability to manipulate the behavior of insect populations in the field.

Many species of bark beetles use complex blends of pheromones and kairomones in chemically mediated communication (Borden 1985, Byers 1989). Within a blend, structural and optical isomers of various components may be required to elicit optimal behavioral responses, and impart species specificity in the chemical message. For example, optimal response of the western balsam bark beetle, *Dryocoetes confusus* Swaine, occurs with a 9:1 ratio of exo- and endo-brevicornin, while the sympatric species, *D. affaber*, requires a 1:2 blend. Virtually no cross attraction occurs between the two blends (Camacho *et al.* 1993, 1994).

In recent years semiochemical-based management tactics have gained substantial credibility in attempts to manage bark beetle populations in North America (Shea 1995). Basic biological research and the demands of industry have resulted in the development of a “high-tech” semiochemical industry (Banfield 1991). Attractive baits are commercially available for most of the major destructive bark beetle species on this continent (Phero Tech 1996). However, the quest continues for a complete understanding of their semiochemical language (Borden 1995). A prime target in this quest in British Columbia (B.C.) is the spruce beetle, *Dendroctonus rufipennis* Kirby, the most destructive insect pest of western spruce forests (Furniss and Carolin 1977). It and other bark beetles are such a problem to the forest industry, that mandatory monitoring and control efforts are now legislated in the Forest Practices Code (FPC) of B.C. (Anonymous 1994).

Exploring the intricacies of semiochemical-based communication is a compelling topic for research, but the impetus for the commercial development of semiochemicals has come from industrial demands to keep operational pest management costs low (Kydonieus and Berzoza 1982). Semiochemical synthesis, however, is often difficult and expensive, due to complex molecular structures. Additional production costs arise from the need for very high quality in both the chemical and optical purity of synthetic compounds. Formulations used to attain controlled release
of different semiochemicals can also affect production costs, as they must be compatible with the structure of the molecules being released. Finally, the species-specific nature of insect pheromones precludes large sales volumes with attendant economies of scale in production and marketing. Therefore the potential profits from any one product are limited (Putland 1994), making commercial development a challenge.

The demand to keep costs low results in two alternative strategies for semiochemical research and synthesis: 1) pursue new, cheap, easily-formulated compounds that are efficacious in manipulating target insect populations; or 2) produce highly specific, potentially expensive compounds with optimal bioactivity. In either case the goal is to increase the operational efficacy and cost efficiency of semiochemical treatments. These two goals are not always compatible, as the optimal blend of compounds for bioactivity may be too expensive (Camacho 1994), or may simply be unnecessary operationally. This thesis will address the issues of enhanced bioactivity and cost efficiency, with the goal of optimizing the commercial pheromone lure for the spruce bark beetle.

SPRUCE BEETLE BIOLOGY, IMPACT AND MANAGEMENT

The spruce beetle attacks all species of spruce in North America. Pioneering females fly and initiate attack on suitable host material from early May through June, with peak flight occurring in June or July. Spruce beetles can disperse over large distances, with flights exceeding 11 km on flight mills (Chansler 1960). Attacking females bore a gallery in the phloem tissue of a selected host, and emit a blend of semiochemicals (Gries et al. 1988). Both males and females are attracted, resulting in a coordinated mass attack, the inoculation of pathogenic symbiotic fungi (Leptographium and Ophiostoma spp.) in the phloem and xylem tissue (Perry 1991, Reynolds 1992) and subsequent death of the tree.

Larvae feed in the phloem tissue, often forming a common feeding front. Developmental rate is temperature dependent (Sahota and Thompson 1979), and the accumulation of temperature through the season determines the proportion of the population maturing in one year. Dyer and Taylor (1971), found that 22% of the beetle population emerging from windfalls within the forest were in a one year cycle, compared to 65% in logging residue exposed to the sun. Pupation is
induced in the third instar when temperature accumulation drops below 9 day-degrees (per day) above the developmental threshold of 6.1° C (Dyer and Hall 1977).

Most beetles in B.C. overwinter as third instars, pupate and mature the next summer, and overwinter a second time as adults. Beetles in a one year cycle must complete development to adulthood by fall of their first season. All beetles must overwinter as adults before they can reproduce (Humphreys and Safranyik 1993). Dependent upon the population, varying proportions of mature brood beetles emerge and drop to the base of standing trees in the fall, burrow into the duff, or bore into the lower bole and root collar of the tree to overwinter (Knight 1961, Safranyik 1988). A smaller proportion of the population emerges to overwinter from downed timber (McCambridge and Knight 1972).

In B.C. large, overmature white spruce, Picea glauca Moench Voss, Engelmann spruce, Picea engelmannii Parry, and their hybrids, Picea glauca x engelmannii Parry ex Engelm, are preferred hosts (Safranyik 1988). Cool, wet, shady areas are the beetles' preferred habitat. At suboutbreak population levels windthrow protected from the sun by an intact canopy is the primary source of breeding material, but trees stressed by fire, flooding, disease, physical damage, and desiccation are also attacked. At high population levels the beetles attack and kill healthy standing trees.

White and Engelmann spruce tend to grow along stream banks and lake shores, or on rocky hills and slopes in areas of high precipitation (Elias 1987). In northern B.C. spruce grows best in devil’s club, Oplopanax horridus (J.E. Smith) Mig., site associations (Watts 1983), characterized by relatively deep, moist, rich soils, with constant water movement, typically found in riparian zones, along streams in gullies and ravines, or at the bottom of long steep slopes. Annual snow packs tend to be deep and persistent in such sites. In the early spring, canopy temperatures can easily reach 16-20 °C in these sites, causing the trees to break dormancy and begin both respiration and transpiration.

At this time soils are often still frozen or waterlogged with insufficient oxygen to drive oxidative metabolism. In these conditions trees will switch to fermentative metabolism (Taiz and Zeiger 1991), producing ethanol as a result. Ethanol may be a primary attractant for pioneering female spruce beetles, as it indicates hosts that are unable to defend themselves against fungal
inoculation through induced resinosis. Thus even though devil’s club sites are excellent for the
growth of young spruce, they bear the highest incidence of and mortality from spruce beetle activity
(Safranyik 1985). In Alaska a similar situation occurs on north-facing slopes where soils remain
frozen well into spring (Hard et al. 1983, Holsten 1984). The preservation of wide riparian zones
required by B.C.’s new FPC (Anonymous 1994), is of major concern to forest managers, as they
harbor large volumes of timber highly susceptible to spruce beetle attack.

The massive mortality of mature spruce caused by spruce beetle outbreaks often results in
an overall reduction in basal area and a shift to dominance of a site by subalpine fir, *Abies lasiocarpa*
Hook Nutt., commonly called balsam (Veblen et al. 1991). Most of the killed spruce remain standing, with a fall rate of 1.5% per year. Increased light passing through the canopy of
death dead trees induces a release in growth rates of previously suppressed understorey trees. Since
balsam tends to predominate the understorey in mixed spruce balsam stands, it grows to form the
next forest canopy. Eventually mortality caused by the western balsam bark beetle as well as heart
and butt rots, will create gaps in the balsam canopy. These gaps allow spruce to grow into the sub-
canopy, where they grow faster than balsam, resulting in an eventual shift back to spruce
dominance. The dominant spruce tend to be well spaced, or in clusters away from the previous
competition of balsams.

In northern B.C. the old-growth sub-boreal montane spruce forests, and riparian zones in
particular, are important as wildlife habitat (Bunnell and Kremsater 1991), particularly for grizzly
bear, *Ursus arctos horribilis* Ord, furbearers and large ungulates (Coupe et al. 1989). Moose,
*Alces alces* (Clinton), and mountain caribou, *Rangifer tarandus* Seton, concentrate in spruce-
dominated riparian areas in winter (M. Wood, pers comm.). Old-growth spruce are of particular
importance to caribou, providing a base for slow growing arboreal lichen that act as overwintering
forage.

Patches of spruce mortality due to spruce beetle activity are beneficial for caribou, because
there is a short but significant lichen bloom in response to increased light. In addition insectivorous
bird and mammal species will forage on beetle larvae in attacked trees. The resulting snags are of
primary importance to at least 23 species of primary and secondary cavity nesters in B.C.
(Machmer and Steeger 1993). Thus for wildlife managers, retaining spruce in riparian areas is of
primary importance. However, if beetle populations erupt out of riparian zones into surrounding
managed stands, wildlife and forest management immediately come into conflict. This is particularly true in northern B.C. where the forest economy will depend on the harvesting of old-growth forests for many years, and much of the timber is susceptible to the spruce beetle. For example in the Mackenzie Timber Supply Area (T.S.A.) approximately 85% of the 122,620,000 m³ of spruce is > 140 years old, and accounts for 36% of the mature timber (Anonymous 1995).

Spruce beetle outbreaks in B.C. have characteristically occurred following widespread windthrow (Safranyik 1985). This availability of excessive amounts of breeding material must coincide with favorable climate, and possibly other factors. When outbreaks do occur they appear to start simultaneously in many isolated epicenters, which intensify, spread and converge over large areas of forest. With the onset of unfavorable climatic conditions, the population begins to crash, fragmenting and eventually declining back into a small isolated pockets.

At the Regional level outbreak populations tend to persist for 5-10 years, killing thousands of hectares of mature spruce timber. Locally, outbreaks tend to explode and crash over periods of approximately four years (Safranyik 1988). Close examination of an outbreak that killed 12,572,000 m³ of mature spruce in the Prince George Forest Region (Humphreys 1993) in the years 1962-1965, reveals some noteworthy observations regarding beetle management. In 1961 several small, scattered populations of spruce beetle were noted but ignored. In 1962 a general increase in infestation was recorded in nine areas along the Fraser and Crooked Rivers, but still without concern. The increase in infestation from 1961 to 1962 was attributed to fire damage, logging slash and heavy windfall in 1960-61. In 1963 aerial surveys revealed that over 182,000 ha of white spruce were attacked with an estimated 10,137,000 m³ of white spruce >35 cm diameter at breast height (dbh = 1.3 m) killed in this year alone. In 1964 and 1965 the infested area declined to 103,700 and 19,320 ha respectively. In 1966 no new attack was observed.

The above scenario highlights the dramatic outbreak potential of spruce beetle populations, and also the difficulty in accurately assessing trends in current infestations. Since the foliage on attacked spruce is often slow to change color (pers. obs.), aerial surveys may fail to disclose the extent of an infestation. During a 1993 aerial survey, L.E. Maclauchlan and P. Byrne (pers. comm.) noted only 27% of the beetle incidence mapped during previous ground probes.
Thus, any method that can be used to keep beetle populations at suboutbreak levels would be of great use to forest managers. Hazard rating to identify the most susceptible stands so they can be given priority for logging is now recommended in B.C. (Anonymous 1996). There is considerable promise in using semiochemical baits applied at 50 m centers to contain and concentrate suboutbreak spruce beetle populations prior to logging (Shore et al. 1990). However, baiting has not been widely adopted. The currently available commercial product comprises only two components, the female produced pheromone fiontalin (Gries et al. 1988), and the host tree kairomone α-pinene (Dyer and Chapman 1971, Borden 1995). These components are released at 2.5 and 1.7 mg per 24h, respectively (Phero Tech Inc., Delta, B.C.)

OBJECTIVES

The overall objective of this research was to assess the potential for improving the efficacy of semiochemical baits for the spruce beetle by testing known and potentially new compounds for their ability to enhance the attractiveness of frontalin, 1,5-dimethyl-6,8-dioxabicyclo(3.2.1)octane, in trap or tree baits.

Frontalin, has long been known for it’s ability to attract spruce beetles to traps (Kline et al. 1974, Furniss et al. 1976), and induce spruce beetles to attack baited trees (Dyer 1973, 1975; Werner 1993). However, the optimal release rates of frontalin that are necessary to trap spruce beetles or induce attack on trees are unknown. My first specific objective was to assess the effect on trap catches of varying the release rate of frontalin alone in baited multiple-funnel traps (Lindgren 1983).

MCOL, 1-methyl-2-cyclohexen-1-ol, is a relatively recently discovered pheromone for the spruce beetle (Wieser et al. 1991). There is considerable geographic variation in it’s enantiomeric specificity (Borden et al. 1996), but in northern B.C. it has not been tested. The second specific objective of this work was to assess the effect on trap catches of adding optically pure enantiomers of MCOL, or different ratios of enantiomers, to frontalin in northern B.C.

My third specific objective was to assess the interaction between MCOL and it’s structural isomer seudenol, 3-methyl-2-cyclohexen-1-ol, in attracting spruce beetles to multiple-funnel traps, and inducing attack on baited trees. Seudenol is a major pheromone component for the Douglas-fir
beetle, *Dendroctonus pseudotsugae*, (Rudinsky *et al*. 1974, Ross and Daterman 1995), and has shown activity for the spruce beetle (Furniss *et al*. 1976). In the presence of a trace of weak acid or heat (+) - MCOL will isomerize to (-) - seudenol, while (-) - MCOL will isomerize to (+) - seudenol (J.P. Lafontaine, pers. comm.). The reaction reaches a dynamic equilibrium at approximately 30:70, MCOL:seudenol. MCOL baits that had >97% pure MCOL prior to 1993 field tests contained 1-14% seudenol after two months in the field (Setter, unpublished data). Spruce beetles produce both MCOL and seudenol in an approximately equal ratio (Borden *et al*. 1996). However, in field use seudenol increased trap catches of spruce beetles (Furniss 1976), but had no effect on baited trees (Dyer and Lawko 1978, Werner and Holsten 1984).

My fourth specific objective was to employ coupled gas chromatographic-electroantennographic (GC-EAD) analysis (Gries 1995) to determine whether there were any potential new semiochemicals for the spruce beetle, and if so, to test the hypothesis that a blend of frontalin with any or all of the new compounds would be more attractive than frontalin alone in traps or on trees.

**METHODS AND MATERIALS**

**EFFICACY OF KNOWN COMPOUNDS**

Chemical source, purity, release devices and release rates for all chemicals tested in this thesis are given in Table 1.

Exp. 1 and 2, Frontalin Dose

Two 10-replicate, randomized complete block experiments (Exp.) were conducted in the Donna and Carmella Creek drainages, on the west side of Williston Lake at approximately 55° N latitude and 124° W longitude. Forests in this area are at the northern reaches of the Engelmann Spruce Subalpine Fir Biogeoclimatic zone (ESSF) (Coupe *et al*. 1991). Exp. 1 ran from 11 May to 11 June and Exp. 2 ran from 11 June to 11 July, 1992. Twelve-unit multiple-funnel traps (Lindgren
1983) were placed at least 15-20 m apart in a line along the forest margin in clearcut areas, or along rights-of-way. Treatments comprised one unbaited control trap, and five baited traps each with a

Table 1. Description of source, purity, release system and release rate for chemicals used in trapping and tree-baiting experiments for the spruce beetle.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source* chemical purity</th>
<th>Optical and Exp. no.</th>
<th>Release device* Release rate (mg/24 h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±) - frontalin</td>
<td>&gt; 97% chem.</td>
<td>1,2</td>
<td>PVC flex lure, 0.8 cm long 0.06</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>10-15</td>
<td>PVC flex lure, 1.0 cm long 0.1</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1,2</td>
<td>PVC flex lure, 7.5 cm long 0.6</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1,2</td>
<td>PVC flex lure, 15.0 cm long 1.2</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1,2</td>
<td>polyethylene Eppendorf tube, 250 µl 2.5</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1,2</td>
<td>2 polyethylene Eppendorf tubes, 250 µl 5.0</td>
</tr>
<tr>
<td>(±) - MCOL</td>
<td>&gt; 95% chem.</td>
<td>6,15</td>
<td>bubble cap, low release rate 0.5</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>3-8</td>
<td>bubble cap, high release rate 1.5</td>
</tr>
<tr>
<td>(+) - MCOL</td>
<td>&gt; 99% R (+)</td>
<td>10,13</td>
<td>polyethylene bag 0.005</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>10,13</td>
<td>polyethylene bag 0.05</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>10,13,14</td>
<td>bubble cap, low release 0.5</td>
</tr>
<tr>
<td></td>
<td>3,4</td>
<td></td>
<td>bubble cap, high release 1.5</td>
</tr>
<tr>
<td>(-) - MCOL</td>
<td>&gt; 99% S (-)</td>
<td>3,4</td>
<td>bubble cap, high release 1.5</td>
</tr>
<tr>
<td></td>
<td>&gt; 96% chem.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCOL (+):(-)</td>
<td>as above</td>
<td>3,4</td>
<td>bubble cap, high release 1.5</td>
</tr>
<tr>
<td>(±) - seudenol</td>
<td>&gt; 98% chem.</td>
<td>6</td>
<td>bubble cap, low release 0.5</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>5-8</td>
<td>bubble cap, high release 1.5</td>
</tr>
<tr>
<td>Chemical</td>
<td>Source</td>
<td>Optical and chemical purity</td>
<td>Exp. no.</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------</td>
<td>----------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>ethyl crotonate</td>
<td>A</td>
<td>&gt; 96% chem., mostly (E)</td>
<td>12,13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>hexanal</td>
<td>A</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>3,3-MCH-ol</td>
<td>S</td>
<td>&gt; 97 % chem.</td>
<td>111,13-15</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td></td>
<td>111,13-15</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td></td>
<td>111,13-15</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>3,3-MCH-one</td>
<td>S</td>
<td>80% chem</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>16% seudenone</td>
<td>9</td>
</tr>
<tr>
<td>seudenone</td>
<td>S</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>α-pinene</td>
<td>P</td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>

*aSymbols for sources as follows: P=Phero Tech Inc. Delta, B.C.; A=Aldrich Chemical Co., Milwaukee, WI; S=Simon Fraser University, Department of Chemistry.

*bLow and high release rate bubble caps have nylon and polyethylene backing, respectively. 3,3-MCH-ol and 3,3-MCH-one were synthesized by Dr. S. Jayaramen, in Dr. A.C. Oehlschlager's lab (S). Ms. L. Gonzalez (S) formulated compounds for release from polyethylene bags.

*cRelease rates determined at ambient lab temperature (P and S).
dispenser releasing (±) - frontalin at 0.06, 0.6, 1.2, 2.5, and 5.0 mg per 24 h (Phero Tech Inc., Delta, B.C.). Baits used in Exp. 2 were recovered from Exp. 1 and re-randomized within each block. Captured beetles were identified to species, sexed, and counted in the field or stored frozen for later assessment.

Exp. 3 and 4, MCOL Enantiospecificity

Exp. 3 and 4 were conducted with the same trap deployment, on the same dates and in the vicinity of Exp. 1 and 2, respectively. Both experiments had 10 replicates and were laid out in a randomized complete block design, with Exp. 4 re-using baits recovered from Exp. 3. In each replicate there was one unbaited control trap, and six traps each releasing (±) - frontalin at 1.2 mg per 24 h. This release rate is approximately half that of commercial baits and was chosen to allow any enhancement effect of MCOL to be revealed (Borden et al. 1996). Five of the frontalin-baited traps in each replicate had (i) - MCOL, (+) - or (-) - MCOL, or 75:25 or 25:75 enantiomeric blends added to the bait. Captured beetles were handled as for Exp. 1 and 2.

Exp. 5-8, MCOL - Seudenol Interaction

Experimental sites were located in the Philips Forest Area of Fletcher Challenge Canada’s Mackenzie Woodlands Division (approximately 53.5° N latitude, and 124° W longitude). Three 10-replicate trapping experiments, with trap type, deployment and processing of collected beetles as above, were conducted between 6 June and 30 August, 1993. In Exp. 5 treatments were an unbaited control trap and four baited traps each releasing (±) - frontalin at 1.2 mg per 24 h. Three of the frontalin-baited traps in each replicate were also baited with (±) - MCOL, (±) - seudenol, or both released together. Exp. 6 tested the effect of combining frontalin with (±) - MCOL and (±) - seudenol at high or low release rates (1.5 and 0.5 mg per 24 h respectively), in four treatments: both at high rates, both at low rates, or with each combination of high and low rates. Exp. 7 tested the attractiveness of (±) - MCOL and (±) - seudenol released alone or together (each at 1.5 mg per 24 h) in the absence of frontalin, compared to an unbaited control trap.

A 20-replicate randomized complete block tree-baiting experiment (Exp. 8) was set up during the week of 10 - 17 May, 1993, in standing timber adjacent to Exp. 5-7. The same bait
treatments as in Exp. 5 were used, but with the baits stapled to mature, dominant spruce trees at least 50 m apart, 10 replicates each in cutting permits (CP) 188-266 and 189-267 (Fletcher Challenge Canada, Mackenzie Woodlands). Baits were placed at maximum reach from the ground on the north side of the tree. Control trees were left unbaited. The mean diameter at breast height (dbh = diam. at 1.3 m) ± SE of the selected trees was 62.5 ± 7.6 cm.

Tree bait efficacy was measured by two factors: 1) attack density (entrance holes per m² of surface area); and 2) attack intensity (spillover to trees within 10 m radius). Baited and control trees were assessed on 27 - 28 July 1993, by counting all spruce beetle entrance holes in a 1.0 m wide strip around the entire bole of the tree between 0.5 and 1.5 m above the root collar. Spillover attack on neighboring trees was assessed by classing all susceptible spruce trees (>20 cm dbh) within a 10 m radius of the baited tree as: 1) no attack; 2) low attack (<20 entrance holes on bole); or 3) mass attack (>20 entrance holes on bole).

**POTENTIAL EFFICACY OF NEW COMPOUNDS**

**Collection of Volatiles and GC-EAD Analysis**

Spruce beetles were obtained from field-infested Engelmann spruce collected near Goldbridge, B.C. All beetles were allowed to emerge in screen cages at approximately 24°C. Males and females were held separately at room temperature between layers of moist tissue paper in glass jars for up to 5 days. Female beetles were then introduced into Engelmann spruce bolts from a windthrown tree collected from near Goldbridge, B.C. Holes 0.5 cm in diameter were bored in the bark to entice females to bore. After gallery initiation a gel cap was placed over the entrance of the gallery to collect frass produced by the boring female.

Frass was collected and placed into a glass tube for airation. A stream of air was drawn over the frass and the volatiles were collected on Porapak Q traps. Trapped volatiles were desorbed with double distilled pentane, concentrated to 5.0 ml with a nitrogen airstream, and analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD) analysis (Arn et al. 1975) adapted for intact bark beetles (Gries 1995). All GC-EAD analyses were done by R. Gries, Chemical Ecology Research Group, S.F.U. GC employed a Hewlett Packard 5840 A instrument equipped with a DB5 coated-fused silica column (30 m x 0.32 mm ID; J & W Scientific, Folson,
Antennal responses were amplified utilizing a custom-built amplifier with a passive low pass filter and a cutoff frequency of 10 kHz. Compound identities were confirmed by comparison of their mass spectra and retention times with those of authentic samples.

Exp. 9-15, Evaluation of Antennally-Active Compounds

Five compounds antennally active in GC-EAD analysis were tested for their ability to alter the response of spruce beetles when they were added to spruce beetle baits comprising frontalin and α-pinene (Table 1) in Exp.9. This experiment was conducted near Coalmont, B.C., between 22 July and 7 August, 1993, with trap type, deployment and processing of collected beetles as above. Treatments included an unbaited control trap, spruce beetle baits (frontalin and α-pinene released together), and spruce beetle baits in combination with each of the following: 1) ethyl crotonate, [(E)-2-butenoic acid ethyl ester], at 0.5 and 5.0 mg per 24 h; 2) 3,3-MCH-ol [3-methyl-3-cyclohexen-1-ol], at 0.5 and 5.0 mg per 24 h; 3) 3,3-MCH-one [3-methyl-3-cyclohexen-1-one] at 0.04 and 0.5 mg per 24 h; 4) hexanal at 0.45 and 4.8 mg per 24 h; and 5) seudenone [3-methyl-2-cyclohexen-1-one] at 0.5 and 5.0 mg per 24 h (Table 1).

(+) - MCOL, and two compounds identified as possible new pheromones in Exp. 9 were tested in 1994 for their ability to enhance response to frontalin in traps in the Granite Creek area near Princeton, B.C. Randomized complete block experiments were set up, with trap type, deployment and processing of collected beetles as above. Treatments were frontalin alone released at 0.1 mg per 24 h, and in combination with: (+) - MCOL at 0.005, 0.05 and 0.5 mg per 24 h (Exp. 10); 3,3-MCH-ol at 0.005, 0.05 and 0.5 mg per 24 h (Exp. 11); ethyl crotonate at 0.05, 0.5 and 5.0 mg per 24 h (Exp. 12); and all three compounds together at the above release rates, respectively (Exp. 13). Five replicates were run in each of three time periods: 10 May - 21 June, 21 June - 2 July, and 2 July - 22 July, 1994.

Ten-replicate randomized complete block tree baiting experiments were set up 10 - 17 May, 1994 in the Municipal creek area NE of Penticton B.C., approximately 50° N latitude and 119° W longitude, (Exp. 14), and on 2 - 7 April, 1994, in CP 403-2333, in Fletcher Challenge Canada’s Clearwater Creek area, NW of Mackenzie, B.C., approximately 56° N latitude and 123° W longitude (Exp. 15). Baits were placed as in Exp. 8. Treatments were frontalin alone released at 0.1 mg per 24 h, frontalin with MCOL at 1.5 mg per 24 h, frontalin with (±) - 3,3-MCH-ol at 1.5
mg per 24 h, frontalin with ethyl crotonate at 2.5 mg per 24 h, and frontalin with all three together at the above release rates, respectively. (+) - MCOL was used in Exp. 14 and (±) - MCOL in Exp. 15, in accordance with the regional differences in bioactivity (Borden et al. 1996; Exp. 3,4). The mean dbh ± SE of selected trees was 56 ± 0.97 cm.

STATISTICAL ANALYSIS

Data from all experiments (except Exp. 9) were transformed using log_{10}(x + 0.5), to adjust for non-normal distribution and heteroscedasticity (Zar 1984), and analyzed by 2-way ANOVA (replicate and treatment) and the Bonferroni t-test (SAS Institute Inc. 1990). Data from Exp. 9 were transformed by log_{10}(x+1), analyzed by 2-way ANOVA (replicate and treatment) and the Ryans Q test (Day and Quinn 1984). In Exp. 14 severe woodpecker activity precluded observation of beetle entrance holes in the bark of some baited trees which were deleted from the analysis. All means and standard errors reported are for the raw data. In all cases $\alpha = 0.05$.

RESULTS AND DISCUSSION

EFFICACY OF KNOWN COMPOUNDS

No significant differences were noted in Exp. 1 in the catches of male or female spruce beetles to increasing doses of frontalin from 11 May to 11 June (Table 2). However, from 11 June to 11 July traps in Exp. 2 with the highest release rate of 5.0 mg per 24 h, caught significantly more females than unbaited control traps (Table 2), while catches of females at all other doses were intermediate between those at the highest dose and that to the unbaited control. Males were unresponsive to frontalin at any dose in either experiment. These results confirm those of Dyer (1971), Kline et al. (1974) and Holsten (1984), who also found that in traps frontalin was moderately attractive alone to spruce beetles. Unlike the results of the above investigators, in my experiments males were unresponsive.

My findings indicate a temporal nature in the response to frontalin, and suggest that early-flying host-seeking beetles may be more responsive to host compounds that are relatively more
Table 2. Response of spruce beetles in Exp. 1 (11 May - 11 June, 1992) and Exp. 2 (11 June - 11 July, 1992) to multiple-funnel traps baited with frontalin released at five different release rates. Blackwater forest area, Fletcher Challenge Canada, Mackenzie Woodlands Division.

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Treatment</th>
<th>Release rate (mg/24h)</th>
<th>No. replicates</th>
<th>No. beetles captured (x ± S.E.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>females</td>
</tr>
<tr>
<td>1</td>
<td>unbaited</td>
<td>-</td>
<td>9</td>
<td>3.0 ± 1.8a</td>
</tr>
<tr>
<td></td>
<td>frontalin</td>
<td>0.06</td>
<td>6</td>
<td>5.2 ± 2.4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>6</td>
<td>3.3 ± 1.1a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2</td>
<td>8</td>
<td>1.9 ± 0.8a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>10</td>
<td>3.2 ± 1.2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>9</td>
<td>10.3 ± 6.5a</td>
</tr>
<tr>
<td>2</td>
<td>unbaited</td>
<td>-</td>
<td>10</td>
<td>2.1 ± 0.8a</td>
</tr>
<tr>
<td></td>
<td>frontalin</td>
<td>0.06</td>
<td>7</td>
<td>7.9 ± 2.4ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>8</td>
<td>9.9 ± 3.8ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2</td>
<td>8</td>
<td>5.8 ± 1.9ab</td>
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<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>10</td>
<td>10.0 ± 5.1ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td>10</td>
<td>13.4 ± 4.7b</td>
</tr>
</tbody>
</table>

*Means within a column and experiment followed by the same letter are not significantly different, Bonferroni t-tests, \( P < 0.05 \).
abundant than pheromones in the early part of the season. The results of Exp. 2 suggest that frontalin may be used by responding females as an aggregation pheromone, and that a sex pheromone function may be served by some other compound. Alternatively, although frontalin alone can induce attack on baited trees (Dyer and Chapman 1971), a synergistic blend of frontalin with a host kairomone like α-pinene (Dyer and Chapman 1971) or with another pheromone may be necessary in traps to induce a high level of response.

In Exp. 3 early in the flight season, the response of males was significantly increased when MCOL of any enantiomeric composition was added to frontalin, while only (+) - MCOL enhanced the attraction of females (Fig. 1). In Exp. 4 later in the flight season, the responses by both sexes were greatly enhanced by MCOL of any enantiomeric composition, up to 4.5 times for females and 7.4 times for males (Fig. 2). The latent time effect evident for frontalin (Table 2) was also evident for female spruce beetles responding to MCOL (Figs. 1,2), supporting the hypothesis that early-season females are more responsive to host kairomones than to pheromones.

The greater response by males than females early in the season (Fig. 1) is consistent with the hypothesis that MCOL functions more as a female-produced (Borden et al. 1996) sex pheromone than as an aggregation pheromone, although it serves the latter function as well. Because MCOL is unattractive alone (Wieser et al. 1991), and the 1.2 mg per 24 h release rate chosen for frontalin was below the threshold for independent attraction (Table 2), the high responses when frontalin and MCOL were tested together, particularly in Exp. 4 (Fig. 2), can definitely be considered to be synergistic.

Although females responded significantly only to (+) - MCOL in Exp. 3 (Fig.1), no preference for any enantiomeric composition occurred in Exp. 4 (Fig. 2). In southcentral B.C., spruce beetles responded positively only to (+) - MCOL and were repelled by the antipode (Borden et al. 1996). In Alaska they responded preferentially to (+) - MCOL and less so to (±) - MCOL, and in southeastern B.C. and northern Alberta they responded with no enantiomeric preference. The lack of any such preference in Exp. 3 and 4 (Figs. 1, 2) places the population in northcentral B.C. as closely aligned with those in the latter two locations. If MCOL is to be used operationally in northcentral B.C., and probably further north as well, (±) - MCOL which is relatively inexpensive, would be the preferred material. Because of the pronounced geographic difference in responsiveness based on the chirality of MCOL (Borden et al. 1996), it would be wise to establish
Response of spruce beetles in Exp. 3 to multiple-funnel traps baited with frontalin released at 1.2 mg per 24 h alone and in combination with MCOL of enantiomeric composition released at 1.5 mg per 24 h. Blackwater forest area, Fletcher Challenge Canada, Mackenzie Woodlands Division, 11 May - 11 June, 1992. Bars for each sex with the same letter are not significantly different, Bonferroni t-tests, $P < 0.05$. 
EXP. 3

<table>
<thead>
<tr>
<th>Treatment &amp; Number of Replicates</th>
<th>Number of Beetles Caught (x + SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNBAITED CONTROL (10)</td>
<td></td>
</tr>
<tr>
<td>FRONTALIN (10)</td>
<td></td>
</tr>
<tr>
<td>FRONTALIN, 97% (+)-MCOL (10)</td>
<td></td>
</tr>
<tr>
<td>FRONTALIN, 75% (+)-MCOL (9)</td>
<td></td>
</tr>
<tr>
<td>FRONTALIN, (±)-MCOL (9)</td>
<td></td>
</tr>
<tr>
<td>FRONTALIN, 75% (-)-MCOL (10)</td>
<td></td>
</tr>
<tr>
<td>FRONTALIN, 97% (-)-MCOL (10)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2  Response of spruce beetles in Exp. 4 to multiple-funnel traps baited with frontalin released at 1.2 mg per 24 h alone and in combination with MCOL of variable enantiomeric composition released at 1.5 mg per 24 h. Blackwater forest area, Fletcher Challenge Canada, Mackenzie Woodlands Division, 11 June - 11 July, 1992. Bars for each sex with the same letter are not significantly different, Bonferroni t-tests, $P < 0.05$. 
EXP. 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNBAITED CONTROL</td>
<td>(10)</td>
</tr>
<tr>
<td>FRONTALIN</td>
<td>(10)</td>
</tr>
<tr>
<td>FRONTALIN + 97%(+)-MCOL</td>
<td>(10)</td>
</tr>
<tr>
<td>FRONTALIN +75%(+)-MCOL</td>
<td>(9)</td>
</tr>
<tr>
<td>FRONTALIN + (±)-MCOL</td>
<td>(9)</td>
</tr>
<tr>
<td>FRONTALIN + 75%(−)-MCOL</td>
<td>(10)</td>
</tr>
<tr>
<td>FRONTALIN + 97%(−)-MCOL</td>
<td>(10)</td>
</tr>
</tbody>
</table>

TREATMENT & NUMBER OF REPLICATES

NUMBER OF BEETLES CAUGHT (x + SE)

MALES

FEMALES
the ranges of other pheromone-based "ecotypes" prior to widespread operational use of this pheromone.

In Exp. 5-7 there were no more than two beetles caught in the baited traps for any collection period, precluding any opportunity to assess the interaction between MCOL and seudenol by trapping experiments. The fact that neither seudenol nor MCOL were attractive alone or in combination in traps supports previous findings (Wieser et al. 1991). The positive responses to baited trees in Exp. 8 (Table 3) indicated that there were beetles in the interior of the stands that the traps surrounded, and suggested that local beetle populations were well contained by the baited trees. Very little spillover attack occurred on spruce trees surrounding the baited trees in all reported experiments, precluding any analysis of a spillover effect. This inadvertent demonstration of the power of tree baiting to contain and concentrate populations of spruce beetles, adds support to the already demonstrated efficacy of this tactic (Shore et al. 1990).

All baited trees in both blocks of Exp. 8 were attacked at statistically equal levels by spruce beetles (Table 3), indicating that frontalin released at 1.2 mg per 24 h, approximately half the current operational rate (Phero Tech Inc.), is sufficient to induce attack and may be all that is necessary for efficacious beetle management. Similarly Borden et al. (1992) found that myrcene was unnecessary along with trans-verbenol and exo-brevicomin to induce attack by the mountain pine beetle, Dendroctonus ponderosae Hopkins, and recommended its deletion from operational baits.

This result differs from those in southcentral B. C. and Alaska where MCOL added to frontalin caused higher numbers of trees to be attacked (Borden et al. 1996), and is in agreement with Dyer and Lawko's (1978) conclusion that seudenol had little effect on the attack of baited trees. It is probable that as a supplement to frontalin, MCOL will be effective only when populations are low or highly dispersed. If MCOL is used, my results indicate that its natural isomerism to seudenol should not affect its performance.

POTENTIAL EFFICACY OF NEW COMPOUNDS

GC-EAD analysis of captured volatiles from female spruce beetles revealed the presence of seven antennally-active compounds (Fig. 3). Four were previously known. Frontalin and α-pinene
Table 3. Comparative attack densities in Exp. 8 on living spruce trees baited with frontalin released alone at 1.2 mg per 24 h and in combination with (±) - MCOL released at 1.5 mg per 24 h, (±) - seudenol released at 1.5 mg per 24 h, or both. South Philips forest area, Cutting Permits 188-266 and 189-267, Fletcher Challenge Canada, Mackenzie Woodlands Division. May - July, 1993.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. replicates</th>
<th>Attack density per m² (± S.E.)⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>unbaited</td>
<td>20</td>
<td>0.0 ± 0.0a</td>
</tr>
<tr>
<td>frontalin</td>
<td>19</td>
<td>46.5 ± 7.9b</td>
</tr>
<tr>
<td>frontalin, (±)-MCOL</td>
<td>21</td>
<td>47.5 ± 8.8b</td>
</tr>
<tr>
<td>frontalin, (±)-seudenol</td>
<td>18</td>
<td>36.4 ± 8.2b</td>
</tr>
<tr>
<td>frontalin, (±)-MCOL,</td>
<td>20</td>
<td>52.9 ± 9.6b</td>
</tr>
</tbody>
</table>

(±)-seudenol

*Means followed by the same letter are not significantly different, Bonferroni t-test, P < 0.05.
Figure 3  Flame ionization detector (FID) and electroantennographic detector (FID: spruce beetle antenna) responses to poropak Q extract of frass volatiles from female spruce beetles boring in spruce billets. Chromatography: split injection; DB5 column; 50°C (hold 1 min), 4°C per min to 120°C, 20°C per min to 240°C.
are a synergistic aggregation pheromone and host kairomone, respectively (Dyer and Chapman 1971). Seudenol is known to be produced by female spruce beetles (Vité et al. 1972). Its pheromonal activity is questionable for the spruce beetle (Rudinsky et al. 1974) (Table 3), but it is a proven aggregation pheromone for the Douglas-fir beetle (Ross and Daterman 1995, Mori et al. 1987). A known antiaggregant for the spruce beetle, 3-methyl-2-cyclohexen-2-one (3,2-MCH or seudenone) (Kline et al. 1974) was shown by Rudinsky et al. (1974) to be produced by the spruce beetle. Three potential new pheromones were hexanal, 3-methyl-3-cyclohexen-1-one (3,3 MCH), a known anti-aggregant for the Douglas-fir beetle (Rudinsky and Ryker 1979), and ethyl crotonate. Because spruce beetles produce seudenol (Vité et al. 1972), the alcohol that corresponds to the ketone 3,2-MCH, it was hypothesized that spruce beetles may also produce the corresponding alcohol for 3,3-MCH. Therefore, 3,3-MCH-ol was tested for bioactivity in addition to the other new antennally active compounds.

Two of the new candidate pheromones showed potential in Exp. 9 as new attractive pheromones (Table 4). At a release rate of 0.5 mg per 24 h (but not at 5.0 mg per 24 h), 3,3-MCH-ol significantly enhanced the response to baited traps over that to frontalin with α-pinene (Table 4). Ethyl crotonate at 5.0 mg per 24 h (but not at 0.5 mg per 24 h) raised the number of beetles captured to a level statistically intermediate between that to frontalin with α-pinene alone and with 3,3-MCH-ol added (Table 4).

Hexanal is a green leaf volatile that is repellent to the southern pine beetle, Dendroctonus frontalis Zimmerman (Dickens et al. 1992) and is inactive against the mountain pine beetle, D. ponderosae Hopkins (Wilson et al. 1996). It failed to cause a significant change in the response by spruce beetles to frontalin with α-pinene. Reanalysis of the GC-EAD traces, coupled with analysis of authentic hexanal, indicated that hexanal was in fact incorrectly chosen for inclusion in this experiment due to a misalignment of peaks in the GC-EAD traces (Fig 3); the antennal response assigned to hexanal was in fact to an unknown compound eluting just before hexanal. The lowest numbers of beetles captured were in traps releasing 3,2-MCH at 5.0 mg per 24 h, apparently in agreement with the results of Furniss et al. (1974), and Kline et al. (1974) that this compound is an antiaggregant for the spruce beetle.

In Exp. 10-13 only ethyl crotonate significantly increased attraction of males and females to traps when added at increasing doses to frontalin (Fig. 4), indicating positively that it is a
Table 4. Response of spruce beetles in Exp. 9 to multiple funnel traps baited with frontalin and \( \alpha \)-pinene alone or with five antennally active compounds, each released at low and high release rates. Slate Creek, near Coalmont, B.C. 22 July - 7 August, 1993, \( N = 10 \) replicates.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Release rate (mg/24 h)</th>
<th>No. beetles captured (( \bar{x} \pm S.E. ))*</th>
</tr>
</thead>
<tbody>
<tr>
<td>unbaited</td>
<td>-</td>
<td>0.4 ± 0.2c</td>
</tr>
<tr>
<td>frontalin, ( \alpha )-pinene</td>
<td>1.2, 2.5</td>
<td>4.4 ± 2.1bc</td>
</tr>
<tr>
<td>+ ethyl crotonate</td>
<td>0.5</td>
<td>3.7 ± 1.0bc</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>8.4 ± 3.2ab</td>
</tr>
<tr>
<td>+ 3,3-MCH-ol</td>
<td>0.5</td>
<td>12.9 ± 4.5a</td>
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<td></td>
<td>5.0</td>
<td>2.1 ± 1.3bc</td>
</tr>
<tr>
<td>+ 3,3-MCH</td>
<td>0.02</td>
<td>2.3 ± 1.0bc</td>
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<td>0.5</td>
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<tr>
<td>+ hexanal</td>
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<td>2.7 ± 1.2bc</td>
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<td>4.8</td>
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<tr>
<td>+ 3,2 - MCH</td>
<td>0.5</td>
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<td>5.0</td>
<td>0.2 ± 0.2bc</td>
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*Means followed by the same letter are not significantly different, Ryans-Q test, \( P < 0.05 \). Results pooled by sex, as catches were low and both sexes responded in a similar manner to all treatments.
Figure 4  Response of spruce beetles to multiple-funnel traps baited with frontalain released alone at 0.1 mg per 24 h and in combination with (+) - MCOL (Exp. 10), (+) -3,3-MCH-ol (Exp. 11), ethyl crotonate (Exp. 12), or all 3 together (Exp. 13) released at 3 increasing rates. Granite Creek forest area Weyerhaeuser Canada, Princeton Woodlands Division. Five replicates each from 10 May - 21 June, 21 June - 2 July, 2 July - 22 July, 1994. Bars within an experiment for each sex with the same letter are not significantly different, Bonferroni t-tests, $P < 0.05$. 
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<tr>
<td>0.1 (14)</td>
<td>a</td>
<td>a</td>
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<tr>
<td>+ (+)-MOOL 0.005 (14)</td>
<td>a</td>
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<tr>
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</tr>
<tr>
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<td>a</td>
<td>a</td>
</tr>
<tr>
<td>+ 3,3-MCH-OL 0.005 (15)</td>
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<tr>
<td>0.1 (15)</td>
<td>a</td>
<td>a</td>
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<tr>
<td>+ ALL THREE 0.005 (15)</td>
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<td>0.05 (15)</td>
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<td>a</td>
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**TREATMENT, DOSE (mg/24h) & NUMBER OF REPLICADES**

**NUMBER OF BEETLES CAUGHT (\( \bar{x} + SE \))**
semiochemical for the spruce beetle. There is no previous record of behavioral activity of ethyl crotonate for any bark beetle (Borden 1985, 1995). In contrast the responses when 3,3-MCH-ol was added to frontalin failed to confirm the bioactivity indicated in Exp. 9 (Table 4). However, there was a strong suggestion of a response by males at a release rate of 0.5 mg per 24 h. A similar increased response to the low dose of 3,3-MCH-ol was observed in Exp. 9, but trap catches were pooled by sex due to low numbers and even ratios of beetles. At an optimal dose, 3,3-MCH-ol may function in a similar manner as MCOL, attracting proportionally more male than female spruce beetles.

(+)-MCOL also failed to increase the response significantly in southcentral B.C., a result in contrast to that achieved in the same geographic region by Borden et al. (1996). However, there was a strong increase in trap catch as the release rate of (+)-MCOL was increased, suggesting a dose dependent response. In Exp. 10 and 13 the release rate of (+)-MCOL was less than half that used by Borden et al. (1996), and one third of the dose used in Exp. 3, 4, 14, and 15. When all three compounds were released together with frontalin the increase in trap catch was again not significant for either males or females (Fig. 4).

On baited trees in Exp. 14 the ternary blend of (+)-MCOL, 3,3-MCH-ol and ethyl crotonate added to frontalin was the only treatment that raised the attack density to a level significantly greater than that induced by frontalin alone (Fig. 5). Quite possibly the 2.5 mg per 24 h release rate of ethyl crotonate in this experiment was too low to induce any effect over that induced by frontalin alone. Because none of the binary blends was tested it is impossible to determine whether the same effect would have been achieved with one of the three components deleted. However, 3,3-MCH-ol was not attractive at release rates > 0.5 mg per 24 h (Exp. 9, 12). In Exp. 14 it was released at a rate of 1.5 mg per 24 h, which may have negated its attraction.

Exp. 15 in northern B.C. revealed no differences between treatments, possibly because the attack induced by frontalin was so great (Fig. 5). However, 3,3-MCH-ol induced a strong but not significant increase in beetle attack density on baited trees (Fig. 5). Heavy woodpecker activity in this experiment made it impossible to determine the attack density on many trees, resulting in a loss of statistical power due to unequal cell sizes. The increased response is possibly noteworthy, as no other compounds added to frontalin have produced such an increase to baited trees in this region. The release rate of 1.5 mg per 24 h was greater than the maximum rate found to be attractive in
Figure 5  Comparative attack densities in Exp. 14 and 15 on living spruce trees baited with frontal in released alone at 0.1 mg per 24 h and in combination with (+) - (Exp. 14) or (±) - MCOL (Exp. 15), 3,3 - MCH-ol, or ethyl crotonate released at 0.05, 0.5, and 2.5 mg per 24 respectively, or with all three together at the above respective release rates. Exp. 14 in the Municipal Creek drainage east of Penticton, B.C. Weyerhaeuser Canada, Okanagan Falls Woodlands Division, May - September, 1994. Exp. 15 in the Clearwater Creek drainage, Fletcher Challenge Canada, Mackenzie Woodlands Division, May - September, 1994. Bars within an experiment with the same letter are not significantly different, Bonferroni t-tests, $P < 0.05$. 


TREATMENT & RESPECTIVE
NUMBER OF REPLICATES
FOR EXP. 14 & EXP. 15

ATTACK DENSITY PER m² (x + SE)
Exp. 9 and 11. My results indicate that as noted for MCOL, 3,3-MCH-ol could show regional variation in its attraction to spruce beetle populations. Dose response and enantiomeric ratio experiments may thus be warranted.

CONCLUSIONS

If nothing else, my thesis demonstrates that the spruce beetle is a very difficult subject for research. Its prevalence is so uncertain and its responses to treatments in experiments so variable that several experiments produced no useable results. Others produced results that were not in agreement with previous research. Nonetheless, there are several significant results and conclusions that have emerged from my work. These are as follows.

1) MCOL is shown for the first time to be an aggregation pheromone for the spruce beetle in northern B.C.

2) Because spruce beetles in northern B.C. are similar to populations in southeastern B.C. and northern Alberta in their lack of enantiospecificity to MCOL, they probably belong to the same pheromone-based ecotype as those in the above two populations; in turn the northern B.C. population is apparently distinct from more enantiospecific “ecotypes” in Alaska and southcentral B.C.

3) Because MCOL is proportionately more attractive to male spruce beetles than frontalin, its role may be biased toward a sex pheromone function, while the role of frontalin may be biased toward an aggregation function.

4) There is an apparent increase in the sensitivity of spruce beetles to pheromones as the flight season progresses, possibly indicating that host-seeking emergents are more responsive to host kairomones than late-flying beetles.

5) Seudenol added to frontalin has no apparent effect on the aggregation by spruce beetles on semiochemical-baited trees. It should not be considered further for operational use against the spruce beetle.
6) Frontalin alone at a release rate at least half that in the currently used operational baits is sufficient to induce a high level of attack on pheromone-baited trees. It should therefore be possible to convert many operational applications to a less expensive, single-component frontalin bait.

7) Ethyl crotonate and 3,3-MCH-ol are potential new aggregation semiochemicals for the spruce beetle, but their bioactivity requires further exploration.

Despite these interesting results, there is none that leads to a compelling argument for immediately changing the current operational tree baits, other than to delete α-pinene. A final set of experiments to compare the efficacy of the present commercial spruce beetle bait with frontalin released alone at rates of 0.1, 0.6, 1.2, and 2.5 mg per 24 h, would determine the optimal dose of frontalin and the need for α-pinene.

The possibility that MCOL, ethyl crotonate, or 3,3-MCH-ol will enhance attack when used alone or in binary combination with frontalin against low or highly dispersed spruce beetle populations merits further research. Because of its demonstrated bioactivity in one of two trapping experiments, its availability and its very low cost, ethyl crotonate should have a high priority for further investigation as a supplement for frontalin in operational tree baits.

It may be that the optimal bait to use will depend upon the management tactic being implemented. For example, frontalin alone or in combination with a cheap synergist would be best used for grid baiting cutblocks as a pro-active treatment to contain scattered populations of spruce beetles prior to harvest. In remote areas where access is difficult and single tree treatments are prescribed, a very attractive, expensive multi-component bait might be justified. For example when lethal spruce beetle trap trees are required, falling them increases costs and can also open the canopy, increasing susceptibility to windthrow. Standing baited lethal trap trees would be an effective tool if attack densities on them could be artificially increased with an optimized semiochemical blend. Finally, monitoring traps may have their efficacy enhanced by using frontalin in combination with MCOL, ethyl crotonate, or 3,3-MCH-ol. Increasing the attraction of trap lures would be very useful for log yard and cold deck areas, where there is a need to monitor spruce beetle populations.
While the use of insect pheromones in forest pest management is well established, continued assessment and development of specific applications is needed. For the high tech pheromone industry an effective approach to growth will be to develop new markets for established compounds. The challenge will be to increase the use of pheromones in operational forestry, by enhancing current bark beetle management strategies, and developing new tactics. If adding synergists to frontal for the spruce beetle will make trapping and single tree treatments more effective, increased efficacy may offset the high cost.
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