SEMIOCHEMICAL-BASED COMMUNICATION IN THREE SPECIES OF BARK BEETLES IN SUBALPINE FORESTS OF BRITISH COLUMBIA

by

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THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

December 1993

APPROVAL

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DEGREE: DOCTOR OF PHILOSOPHY

TITLE OF THESIS:

SEMIOCHEMICAL-BASED COMMUNICATION IN THREE SPECIES OF BARK BEETLES IN SUBALPINE FORESTS OF BRITISH COLUMBIA

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December 16, 1993.
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Abstract

The western balsam bark beetle, *Dryocoetes confusus* Sw., and *Pityokteines minutus* (Sw.), colonize *Abies lasiocarpa* (Hook.) Nutt.; they are sympatric in B.C. with *Dryocoetes affaber* (Mann.) which inhabits *Picea* spp. Chemical analyses of volatiles produced by feeding male *D. confusus* and *D. affaber* disclosed (+)-*exo*-brevicomin and (+)-*endo*-brevicomin [(+)EXOB and (+)ENDOB], as the major insect produced compounds. In laboratory olfactometer bioassays and field trapping experiments, *D. confusus* responded best to a 9:1 blend of (+)EXOB and (+)ENDOB. (+)ENDOB is multifunctional. With (+)EXOB, it is synergistic at low proportions and inhibitory at proportions >50 %. Male response was reduced in the presence of (-)EXOB. Trees baited with (±)EXOB or 9:1 blends of EXOB:ENDOB as (+):(±) and (+):(+) combinations were mass-attacked in similar proportions. However, trees baited with the (+):(+) blend had the highest number of attacks per m^2 and were also surrounded by the most attacked trees. The (+):(±) blend was intermediate in attractancy and (±)EXOB was the least attractive bait. Systematic isolation and identification, disclosed two host compounds: (-)-α-pinene and (*R*)-(-)-myrtenal, that enhance the response of *D. confusus* to pheromones. *D. affaber* responded best to a 1:2 blend of (+)EXOB and (+)ENDOB. Tolerance to variation in the ratio of

pheromone components was restricted to blends that comprised >50 % (+)ENDOB. (-)ENDOB was inhibitory; (-)EXOB was inactive. When the 1:2 and 9:1 blends of (+)EXOB:(+)ENDOB were compared, the responses of *D. affaber* and *D. confusus* were highly specific, providing evidence of pheromonal exclusion. I conclude that the combined effect of optical and geometrical isomerism of brevicomin determines both the level of response and the species-specificity of the pheromones in *D. affaber* and *D. confusus*. In laboratory and field experiments *P. minutus* of both sexes responded to volatiles produced by feeding males. Chemical analyses disclosed (-)-ipsenol and (+)-ipsdienol as the major insect produced compounds. Field experiments indicated that (-)-ipsenol is attractive and (+)-ipsdienol inactive. Responses of *D. confusus* and *D. affaber* to their specific pheromones were not affected by the presence of (-)-ipsenol and (+)-ipsdienol.

Dedicated to the loneliness of the two people who have shared this path with me,

My mother, Sra. Susana Vera de Camacho

My wife, Diana Ivette

Acknowledgments

A short visit to another country is always exciting, especially if the language, the culture, and the climate are different. Working or studying in those places is another story. One must adapt to a whole new set of conditions, but perhaps the biggest challenge is coping with the different mentality of people. I think that this kind of experience should be part of the formation of every scientist. For a foreign student every expression of tolerance and sympathy is most appreciated; I consider myself fortunate to have received many of these from my professors and colleagues.

My scientific supervisor Dr. John H. Borden has been an inspiration for me. He generously provided advice and support of all kinds from moral to financial, worked with me in the field and patiently edited my manuscripts. For all that I express my deepest gratitude and admiration. This research was conducted in close collaboration with Harold D. Pierce, Jr., who contributed to this project with his insight and his vast knowledge of chemistry for which I am most grateful. I thank the members of my supervisory committee, Drs. B.S. Lindgren and J.M. Webster for valuable scientific advice, and critical review of my work.

I am grateful to J.P. LaFontaine, Phero Tech Inc., for assistance with slow-release devices; F. Bellavance, SFU, for advice on statistics; H.S. Whitney, Pacific Forestry Centre, for trainning on mycological procedures; T.M. Poland, SFU, for access to unpublished results. D.E. Bright, Biosystematics Research Institute, for identifiying scolytids. My sincerest thanks to R.A. Jaimes, A.L. Pérez, A. Savoie, B. Cinel, B.C. Kostyk, J. Trouiller, I.M. Wilson, R. Kopecky, and R.R. Setter for field assistance. Thanks to the Merritt Forest District and to Weyerhauser Canada Ltd., for cooperation and allowing me to conduct my research in their jurisdictions.

The Instituto Politécnico Nacional-México, the Escuela Nacional de Ciencias Biológicas, and COFAA-IPN granted me a leave of absence for Ph.D. studies. Special thanks are due to E. Villa-Rivera, E.A. Moreno-Burciaga, T. Villegas-Garrido, S. Sánchez-Colón and G. Zuñiga for their institutional support to my studies, and to my sister, Martha S. Camacho for handling my administrative matters in México.

This research was supported by the World University Service of Canada (GCA program), Simon Fraser University (Graduate Fellowships and President's Research Stipend); the Natural Sciences and Engineering Research Council of Canada; and the Science Council of British Columbia.

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1. INTRODUCTION

1.1 THE SUBALPINE FORESTS OF BRITISH COLUMBIA

Subalpine forests are very important natural resources in western north America. They cover large areas, mostly at high elevations in British Columbia, Alberta, and the U.S. Pacific northwest and Rocky mountain states (Coupé et al. 1991).

According to the "Biogeoclimatic ecosystem classification" proposed by Krajina (1969, 1972) and modified by the B.C. Ministry of forests (Schmidt 1977; Pojar et al. 1987), the subalpine forest is typically included in the "Engelmann spruce-subalpine fir zone" (ESSF). However, the subalpine fir-spruce association in which I focused my research, is also dominant in the uplands of the "Sub-boreal spruce zone" (SBS), and occurs throughout the SBS (Meidinger et al. 1991).

The ESSF is the highest elevation forest zone of the southern two thirds of the B.C. interior. The dominant trees of this zone are Engelmann spruce, *Picea engelmannii* Parry, and subalpine fir, *Abies lasiocarpa* (Hook.) Nutt. Spruce is usually the most abundant species in mature stands, but subalpine fir often dominates the forest canopy at high elevations and in some wetter areas (Coupé et al. 1991). In the northern parts of the province, Engelmann spruce is replaced by white spruce, *Picea glauca* (Moench) Voss, or by *Picea engelmannii X glauca* (hybrid whitw spruce) (Whitford and Craig 1918; Pojar and Meidinger 1991). The ESSF ranges in elevation from 900 to 1700 m in the northern locations and from 1200 to 2300 m in southern B.C. Lodgepole pine is a widespread seral species after fire and can be dominant in the driest region of the zone (Coupé et al. 1991).

The SBS ranges in elevation from valley bottoms to 1100-1300 m, often just below the ESSF. In the higher parts of the SBS hybrid white spruce and subalpine fir are dominant climax tree species. *A. lasiocarpa* is replaced by lodgepole pine, *Pinus contorta* var *latifolia* Engelmann, Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, birches, *Betula* spp., and willows *Salix* spp., in dry and warmer areas (Meidinger et al. 1991).

Less frequently, the spruce-subalpine fir timber type exists in the "Interior cedar-hemlock zone" (ICH). In this zone subalpine fir coexists with white, Engelmann, and Lutz spruce or with Roche spruce, *Picea glauca X sitchensis* hybrids. These forests also comprise western red cedar, *Thuja plicata* Donn, western hemlock, *Tsuga heterophylla* (Raf.) Sarg., and grand fir, *Abies grandis* (Dougl.) Lindl. The ICH occurs at elevations of 400-1500 m in southeastern B.C., and at 100-1000 m high in west-central B.C. (Ketcheson et al. 1991).

Difficult and costly access to high elevation areas has traditionally limited harvesting in the subalpine forest, but the always increasing demand for forest products and the intensive use of lower elevation forests have resulted in growing interest in the management of subalpine forests. According to the B.C. Ministry of forests (1992), *Abies lasiocarpa* in the B.C. interior accounted for 8.39 % and *Picea* spp. 18.56 % of all the log production in the fiscal year 1991-92 (volumes are 6 289 000 and 13 904 000 m^3 respectively). During the same period the volume of the most harvested species, lodgepole pine, was 25.96 % of the provincial total. Therefore, the contribution of fir and spruce from subalpine forests is already very important.

1.2 IMPORTANCE AND BIOLOGY OF THE BARK BEETLES STUDIED.

The major pest of the subalpine fir is the western balsam bark beetle, *Dryocoetes confusus* Swaine. Long ago it was recognized as the cause of severe tree mortality (Swaine 1933; Hopping 1946). In British Columbia *D. confusus* has caused timber losses of ca. 14 million m^3 from 1956 to 1965. The Kamloops, Nelson, Prince George and Prince Rupert Forest Regions have a history of extensive and severe tree mortality due to this pest (Cotrell and Fiddick 1962, 1968; Fiddick et al. 1964). In 1991 and 1992 *D. confusus* infested 62 550 and 16 225 ha, respectively, in B.C., revealing an apparent increase in impact which may be due, at least in part, to expanded surveys (Wood and Van Sickle 1992, 1993).

Infestations by *D. confusus* are frequently underestimated due to incomplete surveys (Unger and Stewart 1986). On the other hand the retention of red foliage for up to five years may cause overestimates of newly-attacked trees (Turnquist and Clarke 1992). A detailed survey conducted by Stock (1991) in 204 000 ha in the Bulkley Timber Supply Area (TSA), Prince Rupert Forest Region, indicated that in 1986, 41 900 ha showed some level of mortality by *D. confusus*, with an average annual mortality due to insects and disease of 4.2 m^3/ha (up to 96 m^3/ha in heavily infested stands), resulting in a volume of 175 980 m^3 of killed timber, most

of it by *D. confusus*. Even considering an optimistic 50 % salvage, the volume of timber loss would be in the order of 87 990 m^3/year in one single TSA. The annual allowable cut due to insects and diseases in fir types was 42 500 m^3 (B.C. Ministry of Forests 1981).

D. confusus preferentially attacks freshly downed trees. Blown down material on the edges of cutblocks and inside the forest after windstorms usually contain significant numbers of *D. confusus* that can later disperse and infest standing trees, making this bark beetle a chronic inhabitant of mature to overmature balsam-spruce stands (Stock 1991; Unger and Stewart 1992). However, because it vectors a lethal, wood-staining fungus, *Ceratocystis dryocoetidis* Kendrick and Molnar, *D. confusus* is an aggresive tree killer. In some stands, the beetle selectively kills trees at a rate that fluctuates yearly, continuing until the mature fir component of the stand is depleted (Erickson and Ferris 1993; Unger and Stewart 1992). Under poor regeneration conditions, all the subalpine fir in a stand can be eliminated.

1.2.1 Biology of *D. confusus*.

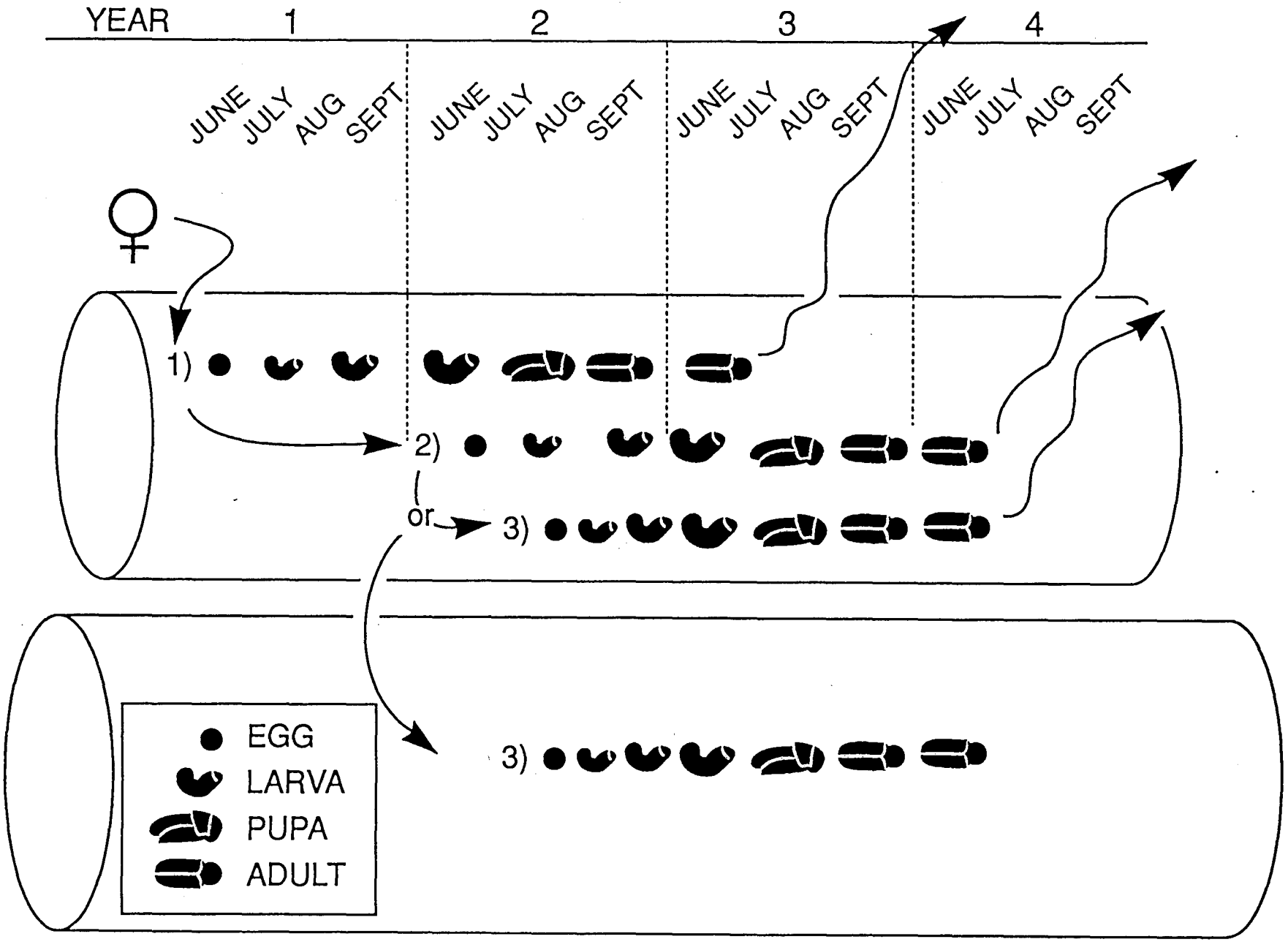
There are seven *Dryocoetes* spp. in North America; five of them, *D. confusus*, *D. affaber*, *D. schelti* Swaine, *D.*

autographus (Ratzeburg), and *D. caryi* Hopkins, can be sympatric in the subalpine forests of British Columbia (Bright 1963). The western balsam bark beetle is the only *Dryocoetes* species capable of colonizing and killing healthy trees.

The life cycle of *D. confusus* varies depending on altitude, latitude and probably climate. It likely takes one year to complete in western and southwestern U.S.A. (USDA 1984), and two yeras in British columbia (Mathers 1931) (Fig 1). Young adults emerge in mid-June and attack new hosts. The pioneer males excavate nuptial chambers and mate with up to four females (Bright 1976). The females bore brood tunnels and lay eggs in them until early fall. The parent beetles then bore feeding tunnels, the male cutting a fresh tunnel from the nuptial chamber and the females continuing in their individual tunnels. The parent adults and young larvae overwinter.

In spring the females extend their tunnels, feed and lay a second brood until early summer (July) when parent beetles either remain in the same host to oviposit again or re-emerge, colonize a second host and produce a third brood. Tunnel excavation and egg laying continue into mid-August; the parent beetles lose their vitality and die by the end of their second winter. Eggs of the first brood

Figure 1. Life cycle of *Dryocoetes confusus*. Two-year model compiled from information in Mathers (1931), Doidge (1981) and Stock (1981). Numbers in parentheses represent broods developing from eggs laid by one mated female. Cylinders depict host trees or logs. Arrows to the right indicate periods of emergence of brood adults.


YEAR
1
2
3
4
JUNE
JULY
AUG
SEPT
1)
2)
or
3)
3)
EGG
LARVA
PUPA
ADULT

apparently hatch in late August, overwinter as small larvae, continue their development through four larval instars, pupate sometime during July-August, develop into callow adults, and overwinter again in the same tree, where they remain until the summer when they emerge. The progeny of the second brood in the same tree and that of the third brood in the same or in another tree presumably follow the same pattern.

Flight habits. Observations conducted in Stanley B.C. (53^{o} latitude) indicate that new adults emerge in late June, and that flight continues throughout July (Mathers 1931). Further North (54.5^{o} latitude) near Smithers B.C., the insect has a bimodal flight pattern with two peaks, one in mid-late June which has an abundance of males, and a second peak in mid-August consisting primarily of re-emergent females (Stock 1991). In the same area traps baited with (±)-*exo*-brevicomin placed 6 m above ground, caught fourfold more beetles than baited traps which were 2 m high (Stock 1991).

Besides the phyto-pathogenic *Ceratocystis dryocoetidis*, other fungi occur in the galleries of *D. confusus*, among them *C. abiocarpa* Davidson (1966), *Pezizella chapmanii* Whitney and Funk, and the entomophagous *Beauveria bassiana* (Bals.) Vuill. (Whitney et al. 1984).

Aggressive or "primary" bark beetle species can attack healthy trees (Rudinsky 1962); they can establish in living trees despite host defenses, typically by an overwhelming mass attack (Raffa and Berryman 1987). By colonizing a tree, primary bark beetles induce physiological changes that allow other organisms to enter the tissues of the attacked tree. "Secondary" or less aggressive scolytids (Rudinsky 1962), nromally do not confront the defensive mechanisms of the host and attack only weakened trees (Craighead et al. 1931; Berryman 1972). These are the extremes of a continuum (Raffa and Berryman 1987); in nature, the population dynamics of both hosts and bark beetles, define the actual type of interaction. Primary bark beetles can remain at low population levels for extended periods of time, infesting only weakened hosts. Environmental disturbances (drought, fire, pollution, etc.), can stress the trees and favor an outbreak of beetle populations (Stark et al. 1968; Mattson and Haack 1987). This often results in massive tree mortality; only then do the beetles decline in numbers and return to the suboutbreak condition (Coulson 1979; Berryman 1982).

For each primary bark beetle species there is a characteristic guild (Pianka 1978) of secondary species. Several species associated with *D. confusus* were listed by

Stock (1981), among them the secondary bark beetle *Pityokteines minutus* (Swaine) (Furniss and Carolin 1977). Although it is often very abundant in trees infested with *D. confusus*, little is known about the biology of this species. Adults usually attack the tops of dying trees or the trunk of suppressed or cut small trees. The males are polygynous; the parental galleries are radiate with four to six or more oviposition galleries originating from the nuptial chamber (Chamberlin 1939; S.L. Wood 1982). The closely related *P. elegans* Swaine is considered to be an important competitor and mortality agent of the fir engraver *Scolytus ventralis* LeConte (Ashraf and Berryman 1969). I hypothesize that a similar situation could exist for the association between *D. confusus* and *P. minutus*.

Another secondary species, *Dryocoetes affaber* (Mannerheim), infests preferentially spruces, *Picea* spp., but is also reported from *Abies*, *Pseudotsuga*, *Larix* and *Pinus* (Furniss and Carolin 1977). It is more widespread than D. *confusus* with wich it is sympatric over much of its range (Bright 1976). *D. affaber* often co-attacks hosts infested with the economically important spruce beetle, *Dendroctonus rufipennis* (Kirby), and has been reported as a competitor of this species (McCambridge and Knight 1972).

1.3 THE ROLE OF SEMIOCHEMICALS IN NATURE WITH PARTICULAR REFERENCE TO THE SCOLYTIDAE (COLEOPTERA).

1.3.1 The ecological role of semiochemicals.

Semiochemicals are chemicals that mediate interactions between organisms (Law and Regnier 1971). I use throughout this work the terminology for the different groups of semiochemicals as defined by Nordlund (1981). I agree with Borden (1985) in considering it appropiate to designate each compound as pheromone, kairomone, synomone, etc., rather than assigning such terms to multicomponent complexes.

Bark beetles are highly specialized organisms that depend on the phloem tissue of trees for food and habitat. As a resource the phloem is limited because it occupies an essentially two-dimensional space, and characteristically exists in suitable trees well scattered throughout in the forest (Atkins 1966). The phloem is covered by a solid barrier of outer bark and protected by an array of physiological defense mechanisms (Berryman and Ashraf 1970; Safranyik et al. 1975; Raffa and Berryman 1983). The chemical communication systems in bark beetles apparently evolved as indicators of potentially suitable sources of food, habitats or mates (Shorey 1973; Borden 1977, 1982;

Schlyter and Birgersson 1989; Raffa et al. 1993). The mass colonization behavior induced by semiochemical signals is probably an adaptive strategy to overcome the defense mechanisms of trees (Rudinsky 1962; Berryman 1974; Birch et al. 1980; Alcock 1982; Raffa and Berryman 1983; Borden 1985).

Natural selection has apparently favored individuals capable of producing and responding to pheromones produced by conspecifics (Burghardt 1970; Whittaker and Fenny 1971; Nordlund and Lewis 1976; Shorey 1977; Nordlund 1981; Alcock 1982; Miller 1990). Species-specificity in pheromonal communication by insects is well documented (Pitman and Vité 1963; Vité et al. 1964; Lanier 1970; Lanier and Burkholder 1974; Shorey 1976; Silverstein 1977; Birch 1978; Payne 1979; Cardé and Baker 1984; Baker 1986; Byers 1989; Linn and Roelofs 1989; Miller 1990; Lewis and Cane 1990). It is of major importance as a mechanism for reproductive isolation, functioning as a pre- or post-mating barrier to hybridization (Wood 1979; Roelofs and Cardé 1974; Vité et al 1978; West-Eberhard 1984; Cardé 1986; Merrill 1991). Mutual inhibition of response to pheromones among North American and European *Ips* spp. seems to be important in maintaining reproductive isolation (Birch and Wood 1975; Kohnle et al. 1986, 1988).

In many species semiochemicals also play a role in the aggregation of individuals (Birch 1984; Borden 1985), and in the prevention of overcrowding (van Lenteren 1981; Prokopy et al. 1984), so that density levels are maintained within an optimal range (Peters and Barbosa 1977; Prokopy 1981). Species-specificity of chemical signals mediates resource partitioning, reducing interspecific competition (Cardé 1986; Miller 1990).

The use of pheromones for resource partitioning is well studied in bark beetles (Svihra et al. 1980; Byers 1983; Kohnle et al. 1986). Many species have clear preferences for discrete areas of a tree, e.g. roots, stump, lower or upper bole, branches of different diameters, or cones, or for particular conditions in those areas, e.g. dead branches, fresh phloem, or fungus-infected tissues (Bright 1976; Blight et al. 1979; Wood 1982). Specific semiochemicals mediate access to and establishment in those particular areas and also function in mutual exclusion of sympatric species (Birch and Wood 1975; Blight et al 1978b, 1979c; Birch et al. 1980; Light and Birch 1982; Kohnle et al. 1988). For example the western pine beetle, *Dendroctonus brevicomis* LeConte, occupies the lower bole and the California fivespined ips, *I. paraconfusus* Lanier, inhabits the crown of the same tree; the presence of insects or synthetic pheromones of the opposite species inhibits the

response of each of these species to its own pheromone (Byers and Wood 1980).

There is also evidence for interspecific cross-atraction to pheromones. In ambrosia beetles the three-dimensional sapwood allows for tolerance or cross atraction among *Gnathotrichus sulcatus* (LeConte), *G. retusus* (LeConte) and the striped ambrosia beetle, *Trypodendron lineatum* (Olivier) (Borden et al. 1980a, 1981). Electrophysiological and field tests with the southern pine beetle, *Dendroctonus frontalis* Zimm., the black turpentine beetle, *D. terebrans* (Olivier), three associated *Ips* species, and some predators and parasitoids, disclose a complex set of interactions ranging from mutual inhibition to cross-attraction (Dixon and Payne 1979; Birch et al. 1980; Svihra et al. 1980; Payne et al 1984; Smith et al. 1990).

Interspecific inhibition and attraction to pheromones probably evolved as secondary traits after insects developed the capacity to recognize species-specific signals. In turn, as speciation proceeds, natural selection should promote departures from an ancestral signal toward new, highly specific pheromones. This trend is apparently evident in regional specificity, chirality, and response to ipsdienol among widely separated populations of pine

engravers, *Ips pini* Say (Lanier et al. 1980; Miller et al. 1989). Moreover, in Wisconsin, *I. pini* and two predators, *Thanasimus dubius (F.)* and *Cylistix cylindrica* (Paykull), have differential preferences for ipsdienol of varying chirality, providing evidence for locally driven adaptations and counteradaptations among predators and prey (Raffa and Klepzig 1989). However, Herms et al. (1991) working only 150 km away, observed very little chiral sensitivity in *T. dubius*.

Interspecific interactions at the semiochemical level clearly influence the spatial and temporal relationships among bark beetles and associated organisms (Wood and Bedard 1977; Herms et al 1991). Thus, they can be a driving force in niche separation (Birch et al. 1980; Paine et al. 1981).

1.3.2 Mechanisms of species-specificity of semiochemical signals

Insects can theoretically achieve specificity in semiochemical-based communication by means of variation in up to three possible factors: chemical composition, time and physiological state.

The simplest mechanism related to chemical composition is variation in the number of semiochemical components, from single to multicomponent signals (Baker and Cardé 1979; Cardé and Baker 1984; Linn et al. 1986; Tumlinson 1988). Individuals in many species require blends of compounds at very precise ratios (Tumlinson et al. 1975; Roelofs et al. 1975; Steck et al. 1982; Bellas and Bartell 1983; Baker 1989). An inactive component may also confer pheromonal specificity. *Z*5-Dodecenyl acetate is produced by female cabbage loopers, *Trichoplusia ni* (Hübner), has no effect on conspecific males, but inhibits the response of male soybean loopers, *Pseudoplusia includens* (Walker), to female cabbage loopers and to their own five-component pheromonal blend (Linn et al. 1984; Grant et al. 1988).

Variation in the stereoisomerism of pheromonal compounds, including geometrical and optical isomerism (Eliel 1962), is a more complex mechanism for semiochemical specificity. Many moths show sensitivity to the *E:Z* isomer ratio (Hecker 1958; Butenandt et al. 1961; Cardé 1986; Linn and Roelofs 1989; Baker 1989). Discrimination among diastereomers is also well documented in the Scolytidae (Silverstein et al. 1968; Renwick et al. 1976; Richerson and Payne 1979; Borden 1985; Byers et al 1989). Chiral sensitivity in insects was first demonstrated with the alarm pheromone of *Atta texana* (Buckley) (Riley et al. 1964). It

is now reported for many species (Silverstein 1979, 1985, 1988; Mori 1984, 1989).

Enantioselectivity is also common in scolytid beetles (Borden et al. 1976, 1980a; Wood et al. 1976; Stewart et al. 1977; Brand et al. 1979; Payne et al. 1982; Birch 1984; Byers 1989; Phillips et al. 1990; Seybold 1992). The pine engraver in California and Idaho produces and responds to (*R*)-(-)-ipsdienol while the antipode is inhibitory (Birch et al. 1980). Combinations of geometrical and optical isomers of pheromone components are known for a number of species. The citrus mealybug, *Planococcus citri* (Risso), responds more to the (*R*)-*cis*-(+)- isomers than to any other stereoisomers of its pheromone (Bierl-Leonhardt et al. 1981); *Pityogenes chalcographus* (L.) responds better to a combination of (2*E*, 4*Z*) methyl 2,4-decadienoate and (2*S*, 5*R*)-chalcogran than to other stereospecific blends (Byers et al. 1989).

Theoretically, the utilization of numerous highly specific pheromone communication channels could be achieved by exploiting combinations of geometrical and optical isomers. However, this level of specialization has been demonstrated only in Khapra beetles, *Trogoderma granarium* Everts; they use the (*R*)-(-)- enantiomers of (*Z*)- and (*E*)-trogodermal in a 92:8 ratio, while beetles in three other

Trogoderma spp., respond to the *R*-(-)- enantiomer of either (*E*)- or (*Z*)-trogodermal (Cross et al. 1976; Silverstein et al. 1980).

Another possible means of varying chemical composition is the use of combinations of pheromones and host kairomones. Such combinations may favor species-specificity between insects as well as between insects and their hosts (Angst and Lanier 1979; Mustaparta et al. 1979; Whitehead 1986; Billings et al. 1976; Borden et al. 1987a; Miller and Borden 1990a, 1990b).

Temporal separation (diel and seasonal) in the use of pheromone channels often contibutes to species-specificity (Brown 1972; Roelofs and Cardé 1974; Shorey 1974; Silverglied 1977; Teal et al. 1978; Hendrickse 1979; Cardé 1986). It can be very important in some lepidopteran families, e.g. Sesiidae and Pterophoridae (Greenfield and Karandinos 1979; Haynes and Birch 1986). Yet, for most species diel and seasonal separation is insufficient to ensure pheromonal-specificity; therefore, temporal variation usually acts simultaneously with other mechanisms, reinforcing the specificity (Sanders 1971; Roelofs and Cardé 1971, 1974; Kaae et al. 1973; Liebheir and Roelofs 1975; Tamaki and Yamaya 1976; Cardé et al 1977; Grant 1977; Miller 1990).

The internal state of the insect can profoundly influence the production and response to semiochemicals. Maturation, nutritional state and endocrine regulation are of major importance (Borden 1967, 1985; Borden et al. 1969, 1986; Riddiford and Williams 1971; Vité et al. 1972; Harring 1978; Byers 1983). For scolytid beetles, it has been suggested that production of specific pheromones is determined in some cases by a selective capacity to convert particular ingested or inhaled precursors into pheromones, a process that can be highly stereospecific (Renwick et al 1976; Byers 1983; Pierce et al. 1987; Vanderwel and Oehlschlager 1987).

At the level of perception and response to pheromones, the existence of olfactory cells with varying degrees of specialization has been documented in some species of moths (Baker 1989) and bark beetles (Payne et al. 1982; Dickens 1986). Western *I. pini* produce and respond to (-)-ipsdienol; they have 12 receptor cells keyed to the (-) enantiomer for each cell keyed to the antipode. Eastern populations respond to a blend of 65 % (+)- and 35 % (-)-ipsdienol and possess roughly 1:1 of each receptor type (Mustaparta et al. 1980, 1985). Behavioral responses to olfactory stimuli very likely result from the integration process in the central nervous system (Lanier et al 1972;

Mustaparta et al 1985; Byers 1989). Thus, differences at the receptor level combined with integration of information in the central nervous system, provide major elements for specificity in chemical communication.

1.3.3 Chemical communication and the systems approach.

Much work has been done in 30 years of pheromone research. Single-component pheromones, rarely, if ever occur in nature. Instead, research has disclosed that semiochemicals act in complex systems (Silverstein and Young 1976; Birch 1984; Tumlinson 1988). Silverstein (1981) describes chemical communication in insects as a language in which complex statements can be made with combinations of chemicals, sent through a number of channels. The language has a vocabulary and syntax, the message has a different meaning for every species and sometimes sex, and depends on the context. Insects call and respond only at particular times under particular circumstances. Birch (1984), and Tumlinson (1988) have pointed out that effective use of semiochemicals in pest management requires an understanding of chemical communication in insects as functional systems.

Most semiochemical research has focused on the study of pheromones of single species. Studies of complex systems involving interspecific interactions have usually centered around economically important pests, e.g. *Dendroctonus brevicomis, D. ponderosae* Hopkins, *D. frontalis, Ips typographus* L. Progress in this research is now allowing the development of novel strategies and tactics for the use of semiochemicals to manipulate natural population interactions among forest insects (Borden 1989, 1992).

1.4 THESIS OBJECTIVES

This study is focused on the semiochemical-based communication of three sympatric species of bark beetles: *Dryocoetes confusus* and *Pityokteines minutus* in subalpine fir and *Dryocoetes affaber* in Engelmann spruce, and initiates the investigation of some of their interspecific interactions. In this context, I expect my research to generate information that may contribute to the knowledge and understanding in ecology and evolutionary biology as well as to the development of ecologically and economically sound tools for pest management.

My specific objectives were:

1) to elucidate further the semiochemical complex of the western balsam bark beetle,
2) to describe pheromone-based communication in *D. affaber*,
3) to initiate a study of semiochemical communication in *P. minutus*,
4) to explore semiochemical interactions between the three species, and
6) to improve the potential for the use of semiochemicals for management of *D. confusus*.

2. STUDIES ON SECONDARY ATTRACTION OF *DRYOCOETES CONFUSUS*

2.1 INTRODUCTION

During the host colonization process, depending on the species, pioneer bark beetles may land at random on trees (Moeck et al. 1981; Wood 1982); or respond to combined visual stimuli and olfactory signals released by the host which constitute "primary attraction" (Pearson 1931; Wood 1982); once pioneers arrive to the tree, there is "secondary attraction" in which insect-produced compounds (pheromones), characteristically act synergistically with host volatiles, eliciting a massive response from conspecifics (Borden et al. 1975; Borden 1982).

Host colonization by bark beetles is a process that can be grouped in phases. My concept is based on the sequences proposed by Wood (1972, 1982), and Borden (1982), but includes five phases: dispersal, selection, concentration, antiaggregation, and establishment. I consider the onset of antiaggregation mechanisms as a distinctive phase.

Research in this section focused on the role of pheromones during the dispersal, selection, and concentration phases. Discovery of the antiaggregative properties of (+)-*endo*-brevicomin (Stock et al. 1990), provide a solid base for further research on the mechanisms used by *D. confusus* to terminate aggregation.

2.2 AGGREGATION PHEROMONES IN *DRYOCOETES CONFUSUS*

2.2.1 Introduction

Semiochemical-based communication in the genus Dryocoetes. Response to semiochemicals by *Dryocoetes* spp. was first observed in the 1970's. Furniss et al. (1976), reported attraction of *D. autographus* and *D. affaber*, to uninfested spruce logs and/or to one or more semiochemicals produced by spruce beetles, *Dendroctonus rufipennis*: *trans*-verbenol, *cis*-verbenol, seudenol and frontalin. Nilssen (1979) documented the first evidence of secondary attraction in the genus; under laboratory conditions male and female *D. autographus* and *D. hectographus* Reitter responded to frass produced by conspecific males. European male *D. autographus* produce *exo*- and *endo*- brevicomin (7-ethyl-6,8-dioxabicyclo [3.2.1] octane), In field tests (±)-*exo*-brevicomin and (±)-*endo*-brevicomin were attractive when offered alone, but the mixture of both brevicomins was less attractive (Kohnle and Vité 1984). Female *D. autographus* responded to (+)-*endo*-brevicomin while the antipode was inactive; the role of *exo*-brevicomin remains inconclusive (Kohnle 1985). *D. hectographus* were caught in traps baited with western pine beetle lures (frontalin, myrcene and *exo*-brevicomin) in a test conducted in China (Miller et al. 1989).

Semiochemicals of D. confusus. Stock and Borden (1983) found evidence of secondary attraction in the western balsam bark beetle. Laboratory and field experiments demonstrated that beetles feeding in logs produce an aggregation pheromone attractive to both sexes. Also they observed response to uninfested logs of *Abies lasiocarpa* and to volatiles of the host tree. Schurig et al. (1983) disclosed that abdominal extracts of males allowed to feed in logs for 24 h contained (+)-*exo*-brevicomin (99.2 % optical purity) and (+)-*endo*-brevicomin (81.6 % optically pure); the ratio of *exo*- to *endo*-brevicomin was approximately 18:1. (Hereafter, I use "EXOB" for *exo*-brevicomin and "ENDOB" for *endo*-brevicomin; blends are referred to as EXOB:ENDOB in that sequence).

Borden et al. (1987) found no EXOB in emergent males; (+)EXOB was present in highest levels in feeding unmated males and declined in amount after contact with females. (+)EXOB was attractive to female beetles in laboratory bioassays and the presence of the antipode did not inhibit the response since (±)EXOB was attractive in both laboratory and field conditions. Field experiments (Stock 1991; Stock et al. 1993) indicated that (±)EXOB is more attractive to *D. confusus* than either enantiomer alone, suggesting a

synergistic and/or additive effect for the unnatural (-) EXOB.

Field tests showed that (±)ENDOB in a 1:1 ratio with (±)EXOB inhibited attraction; (-)ENDOB did not inhibit attack by *D. confusus*. Thus Stock et al. (1990) concluded that (±) or (+) ENDOB was an antiaggregation pheromone.

Other compounds identified in abdominal extracts of feeding males were myrtenol, verbenone, *trans*-pinocarveol, *cis*- and *trans*-p-menthen-7-ol. *trans*-Verbenol was always present in males and in feeding or mated females. In field tests (±)-myrtenol was unattractive by itself, but showed a synergistic effect when combined with (±)EXOB and monoterpenes in traps (Borden et al. 1987). However, the activity of myrtenol remains unverified (J.H. Borden[1], pers. comm.).

The need for further research arose as the prospect of using semiochemicals for pest management for *D. confusus* became viable. Pioneer research found that under field conditions EXOB elicits only weak responses, catches in traps are low compared to those for other bark and ambrosia

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[1]Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby B.C.

beetles and the results are quite variable. Thus, it was apparent that there were some components of the attractive semiochemical blend (either host kairomones or beetle produced pheromones, or both), that remained undisclosed.

Detection of (+)EXOB and (+)ENDOB in early stages of host colonization by *D. confusus* (Schurig et al. 1983), suggested that the role of ENDOB was not restricted to anti-aggregation. Therefore, I hypothesized that ENDOB acts in the aggregation process.

My objectives were:

1. to search for missing components that could enhance the attractiveness of EXOB, the known pheromone of *D. confusus*,
2. to investigate the role of ENDOB as a semiochemical for *D. confusus*, and
3. to determine the role of chirality in the system.

2.2.2 Materials and Methods

Synthetic Pheromones. (±)EXOB (96.3 % pure, 2.5 % ENDOB) and (±)ENDOB (96.4 % pure, 0.4 % EXOB), were obtained from Phero Tech Inc., Delta, B.C. Chiral brevicomins were synthesized by B.D. Johnston (Department of Chemistry, Simon Fraser University), according to procedures developed by Johnston and Oehlschlager (1982), and Oehlschlager and Johnston (1987). Formulations included (+)EXOB (98.1 and 93.05 % chemically and optically pure, respectively), (-)EXOB (97.0 and 95.0 % chemically and optically pure, respectively), (+)ENDOB (98.8 and 90.15 % chemically and optically pure, respectively). Blends of EXOB and ENDOB were prepared on a weight to weight basis.

Collection of Insects and Hosts. Subalpine firs infested with *D. confusus* were felled near Merritt, B.C. and sawn into 70 cm-long bolts, which were placed in cages in the laboratory. Emerging adults were sexed and kept on moistened paper at 5^{o}C until used for laboratory bioassays.

Analysis of Volatiles[2]. To determine the natural ratio of EXOB to ENDOB released into the air, volatiles from fresh subalpine fir bolts infested with male *D. confusus* were captured on Porapak-Q (Byrne et al. 1975). Frass from boring males was collected and steam distilled through a 30-cm glass Dufton column. The volatiles from the infested log aerations and frass were analyzed by gas chromatography (GC) using Hewlett Packard 5830A and 5880A instruments equipped with capillary inlet systems and flame ionization detectors. Samples were analyzed on open tubular glass columns coated with SP-1000 (Supelco, Bellefonte, Pennsylvania). Temperature program was 70°C for 2 min, then 4°C/min to 180°C holding for 20 min. Coupled gas chromatography-mass spectrometry was performed with a Hewlett Packard 5895A GC-MS-DS fitted with a 30 m X 0.32 mm ID fused silica column coated with SP-1000 (J & W Scientific, Inc., Folsom, California). Helium was the carrier gas for GC and GC-MS.

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[2]In this and following sections the chemical procedures [extraction, fractionation, analysis and (where not otherwise acknowledged) synthesis of various semiochemicals], were performed by Dr. Harold D. Pierce, Jr., Department of Chemistry, Simon Fraser University, Burnaby, B.C.

Laboratory bioassays. I used walking beetles in an open arena olfactometer (Wood and Bushing 1963; Stock and Borden 1983). Groups of 10 beetles were exposed for 2.5 min to an air stream (700 mL/min) containing volatile stimuli applied in 10 μL of pentane to a filter paper wick. Fifty healthy beetles of each sex were tested per stimulus. The solvent was used as a control, room lighting was diffuse and room temperature was 20-21^{o}C.

Lab. Bioassay 2A. Determination of the optimal ratio of EXOB:ENDOB for attraction of *D. confusus* was conducted with a series of 1 pg stimuli consisting of the naturally-produced (+)EXOB and (+)ENDOB. Blends of these two compounds at ratios of 9:1, 7:1, 4:1, 2.3:1, and 1:1 were also tested.

Lab. Bioassay 2B. I carried out a dose-response bioassay using the 9:1 blend of (+)EXOB:(+)ENDOB at five doses (0.1, 1, 10, 100 and, 1 000 pg); pentane (10 μL) and (+)EXOB (1 pg) were included as control stimuli.

Lab. Bioassay 2C. In this experiment, I tested 1 pg stimuli of combinations of (+) and racemic (±) materials in the 9:1 blend of EXOB:ENDOB. The (-) enantiomers were not

tested as they were reported to be inactive (Borden et al. 1987: Stock et al. 1990).

Lab. Bioassay 2D. To assess the effect of (-)EXOB in the binary blend, 0.1 pg stimuli of the 9:1 blend of (+)EXOB:(+)ENDOB and of (-)EXOB were tested alone and mixed.

Field experiments. Trapping experiments were conducted in a subalpine forest located 40 km West of Merritt, B.C. In all experiments 12-unit, multiple-funnel traps (Lindgren 1983; Phero Tech Inc., Delta, B.C.), were placed 15 m apart in randomized complete blocks, with from 10 to 20 replicates. As a standard procedure in all field experiments, volatile stimuli were released either as single compounds or in blends, from a glass capillary tube (1.0 mm ID) sealed at one end, the release rate was approximately 0.2 mg/24 h as determined in the laboratory at 20^{o}C (Stock 1991).

Field Experiment (Exp.) 2A. This experiment was performed *before* the laboratory bioassays. In late summer 1990, I tested the attractiveness of (±)EXOB and blends of (±)EXOB and (±)ENDOB at the following ratios: 11:1, 5:1, 1:1.

Field Exp. 2B. This and later experiments were conducted using synthetic (+)EXOB and (+)ENDOB (the

naturally occurring enatiomers). In the summer of 1991, the first of two field experiments tested mixtures of (+)EXOB and (+)ENDOB at three different ratios: 5:1 (the most attractive blend in the previous field experiment with racemic brevicomins); 9:1 (which elicited the best response in laboratory bioassays), and the 1:1 blend, which was unattractive for *D. confusus* (Stock et al. 1990), but attractive in the field for *D. affaber*. Also included were (±)EXOB and an unbaited control.

Field Exp. 2C. Having investigated the role of ratios of geometrical isomers of pheromones in mediating the aggregation response by *D. confusus*, I then tested for enantioselectivity. Because (-)EXOB was known not to be attractive (Borden et al. 1987; Stock et al. 1990), only combinations of (+) and (±) EXOB and ENDOB were tested at a 9:1 ratio.

Field Exp. 2D. In this dose-response experiment conducted in the summer of 1992, the most attractive treatment from previous experiments was tested. I deployed the 9:1 blend of (+)EXOB:(+)ENDOB at 3 different release rates: 0.2, 1.0 and 2.0 mg/day using 1, 5, and 10 glass capillaries respectively, each one releasing the blend at approx. 0.2 mg/day.

Field Exp. 2E. To investigate the role of (-)EXOB further, I conducted a final field experiment near the end of the summer of 1992. I tested the following stimuli: the attractive mixture of (+)EXOB and (+)ENDOB at the 9:1 ratio released at approximately 0.2 mg/day; (-)EXOB:(+)ENDOB at the 9:1 ratio, and also the (+):(+), 9:1 stimulus with another glass capillary containing (-)EXOB in the same trap.

Statistical Analysis. Laboratory bioassay results were analized by one-way analysis of variance (ANOVA) and the Ryan-Einot-Gabriel-Welsch multiple F or "REGWF" test (Schlotzhauer and Littell 1987) utilizing percentages of positive responders converted to $p'=\arcsin\sqrt{p}$, to approximate a normal distribution (Zar 1984). For field trapping experiments I used two-way ANOVA and the REGWF test on numbers of beetles captured transformed by $x'=\log(x+1)$ to remove heteroscedasticity (Zar 1984). If data were not then normally distributed I used the non-parametric Friedman test (Friedman 1937; Conover 1980). In all cases $\alpha=0.05$. I employed SAS computer software (SAS Institute 1990) for the analyses.

2.2.3 Results and Discussion

Analysis of Volatiles. GC analysis (confirmed by mass spectrometry) of volatiles emanating from males in logs disclosed EXOB and ENDOB in a ratio of 11.4:1 (Fig. 2); frass volatiles contained the isomers in a 5.4:1 ratio. Schurig et al. (1983) analyzed crushed abdomens of feeding male *D. confusus* and determined an 18:1 average ratio of EXOB:ENDOB, mostly as (+) enantiomers. The consistent presence of these two compounds from the start of volatile production by male beetles reinforced the hypothesis that they both have a role in aggregation of *D. confusus*, even though high levels of (±)ENDOB were known to inhibit response (Stock et al. 1990). This hypothesis was tested in further experiments. In addition determination of variable ratios of the two compounds in different analyses (see above) necessitated that the optimal ratio be determined experimentally.

Field Exp. 2A. The 5:1 blend of (±)EXOB:(±)ENDOB was most attractive to *D. confusus* females (Fig. 3); males responded poorly and did not discriminate between treatments. On the other hand *D. affaber* responded optimally to the 1:1 blend, lower catches were obtained with the 5:1 blend, and no *D. affaber* responded to the other treatments.

Figure 2. Gas-liquid chromatogram of Porapak Q-trapped volatiles produced by male *Dryocoetes confusus* feeding inside fresh bolts of *Abies lasiocarpa*, showing *exo*-brevicomin and *endo*-brevicomin in an 11.4:1 ratio.

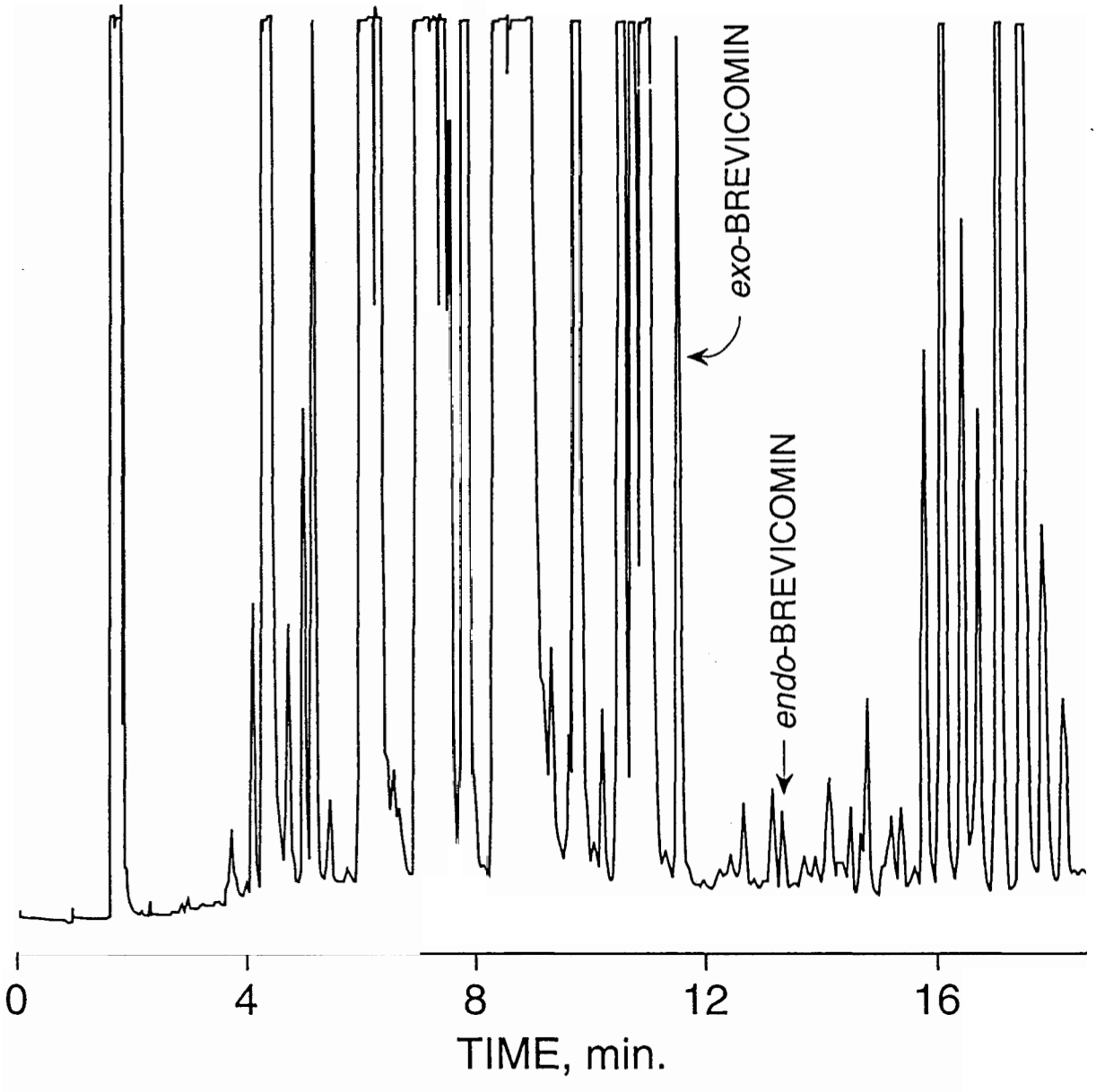
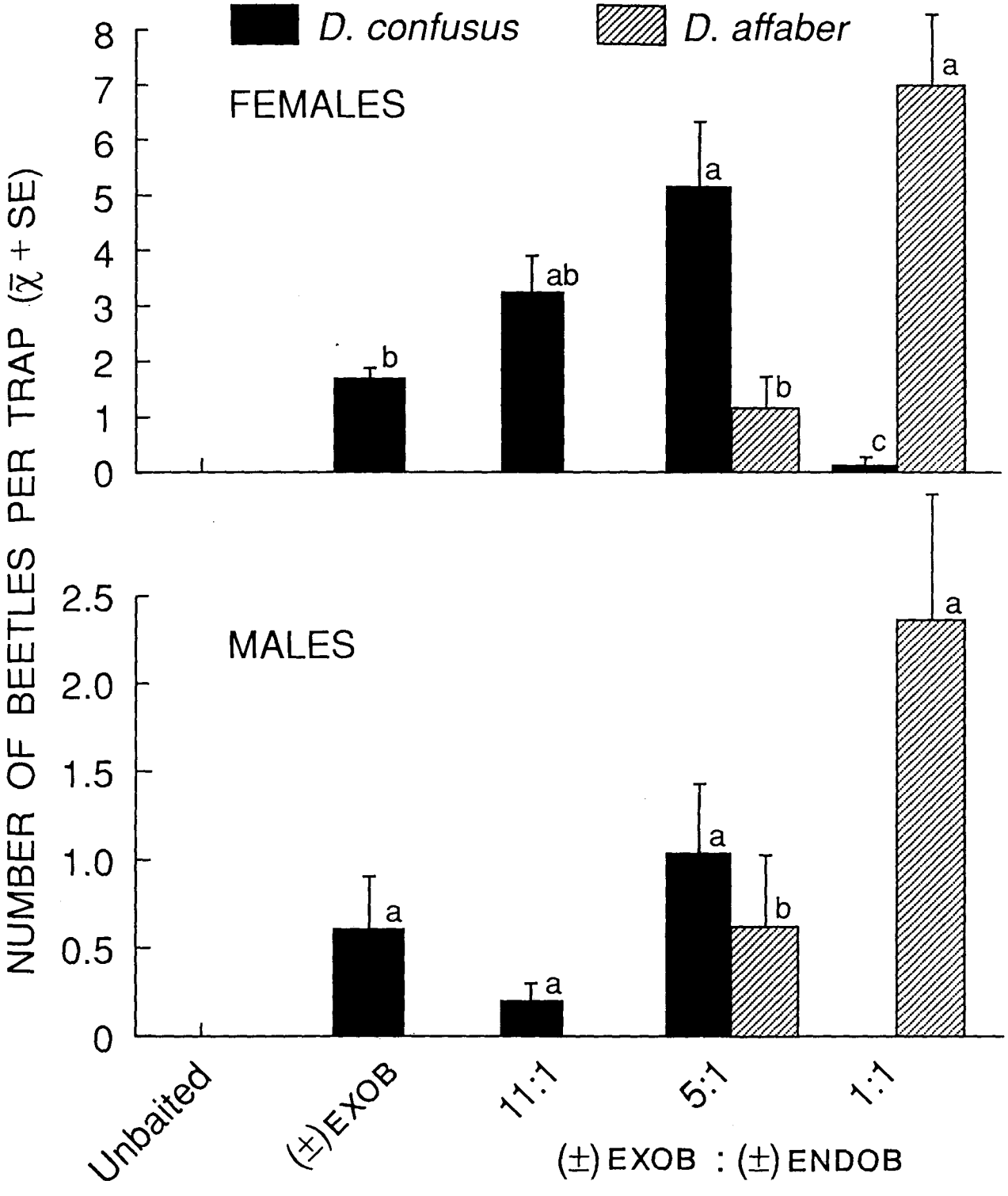

exo-BREVICOMIN
endo-BREVICOMIN
0
4
8
12
16
TIME, min.

Figure 3. Numbers of *Dryocoetes confusus* and *D. affaber* caught in Field Exp. 2A to traps baited with blends of (±)-*exo*-brevicomin (EXOB) and (±)-*endo*-brevicomin (ENDOB) at several ratios, 10 replicates, 25 August to 22 September, 1990. Bars with the same letter within each species are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test on data transformed by $x'=\log(x+1)$, $P<0.05$. Treatments with zero catches were excluded from statistical analysis. More females than males were flying during this experiment.


D. confusus
D. affaber
FEMALES
MALES
NUMBER OF BEETLES PER TRAP (x̄ + SE)
0
1
2
3
4
5
6
7
8
0
0.5
1.0
1.5
2.0
2.5
a
ab
b
a
b
c
a
a
a
b
a
Unbaited
(±)EXOB
11:1
5:1
1:1
(±) EXOB : (±) ENDOB

Lab. Bioassay 2A. The highest response by female *D. confusus* was elicited by the (+)EXOB:(+)ENDOB blend at a 9:1 ratio (Fig. 4). Since field experiment 2A indicated that the 11:1 ratio was less attractive than the 5:1 ratio (Fig. 3), I did not test ratios >9:1. The high responses to picogram level stimuli demonstrate the remarkable sensitivity of *D. confusus* to its pheromones.

Lab. Bioassay 2B. This dose-response experiment further exemplified the sensitivity of *D. confusus* to pheromones. There was a significantly higher response from females to the 9:1 blend at 1 pg than to four other doses (from 0.1 to 1 000 pg), and by all doses of this blend than to (+)EXOB at 1 pg (Table 1). The response by males did not show any statistically significant effect of concentration, but the highest numerical response was also to 1 pg stimuli. Previous studies (Borden et al. 1990; Stock 1991) report the best response of *D. confusus* to (±)EXOB with a stimulus of 5 ng, 5 000 times higher than 1 pg. The marked difference in the concentration required to elicit beetle response indicates that the 9:1 blend is a more complete chemical message for *D. confusus* than (±)EXOB alone.

Figure 4. Response of *Dryocoetes confusus* in Lab. Bioassay 2A to 1 picogram stimuli of (+)-*exo*-brevicomin (EXOB), (+)-*endo*-brevicomin (ENDOB) and five blends of the two isomers in various ratios. Fifty beetles of each sex tested per stimulus. Response to pentane controls: males 4 %; females 6 %. Percents with the same letter are not signficantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$

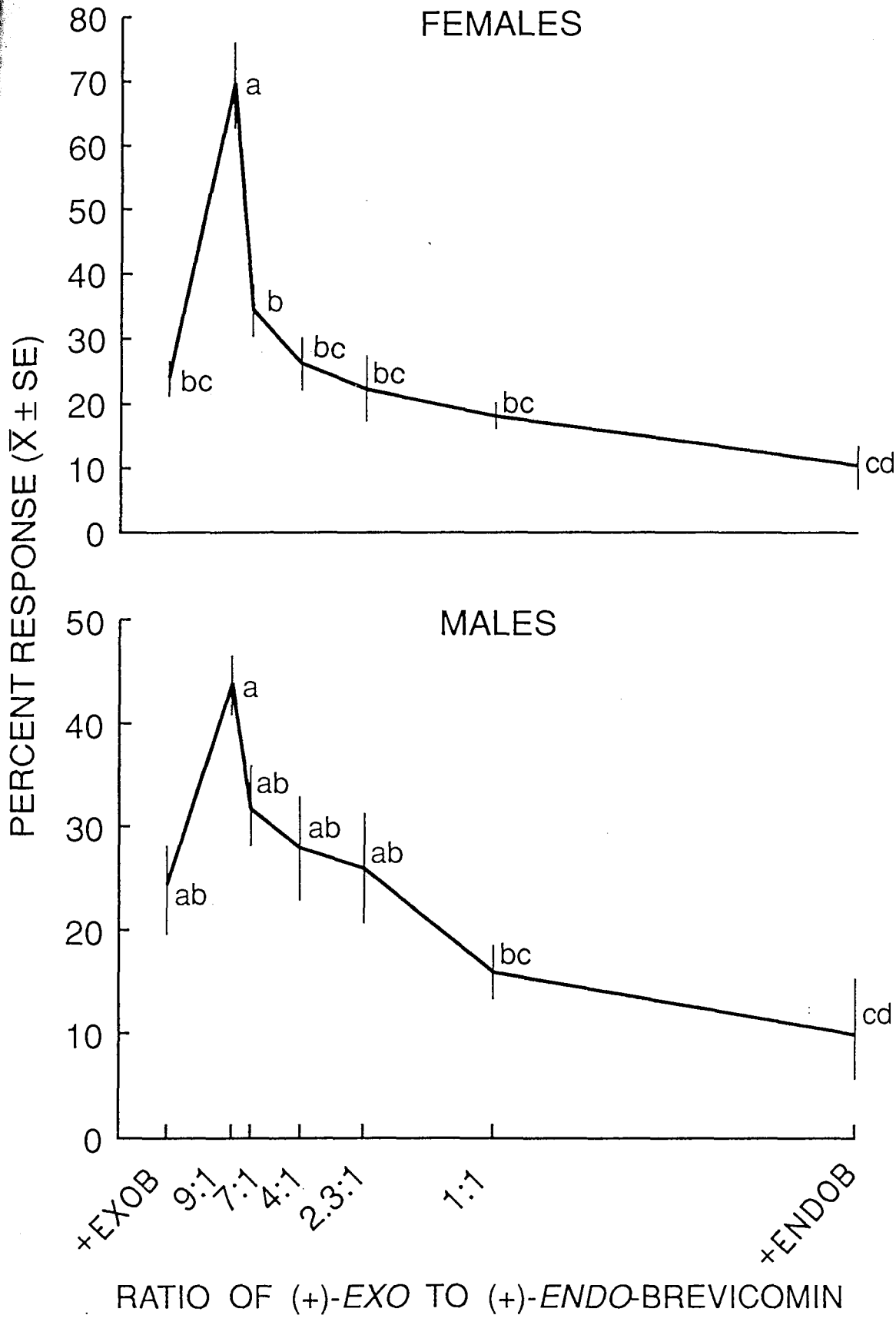

FEMALES
80
70
60
50
40
30
20
10
0
a
b
bc
bc
bc
bc
cd
MALES
50
40
30
20
10
0
a
ab
ab
ab
ab
bc
cd
PERCENT RESPONSE ($\bar{X} \pm SE$)
+EXOB
9:1
7:1
4:1
2.3:1
1:1
+ENDOB
RATIO OF (+)-*EXO* TO (+)-*ENDO*-BREVICOMIN

TABLE 1. Response by *Dryocoetes confusus* in laboratory bioassay 2B to different doses of 9:1 blends of (+)-*exo*-brevicomin (EXOB) and (+)-*endo*-brevicomin (ENDOB).

STIMULUS	DOSE (picograms)	PERCENT RESPONSE ($\bar{X}$±SE)[a] MALES	FEMALES
PENTANE (10 μL)	0	4.0±3.2 c	6.0±2.0 d
(+)EXOB	1	24.0±4.0 ab	24.0±2.4 c
EXOB 9:1 ENDOB	0.1	28.0±3.7 ab	54.0±4.0 b
EXOB 9:1 ENDOB	1	38.0±3.7 a	84.0±2.4 a
EXOB 9:1 ENDOB	10	22.0±3.7 b	68.0±2.0 b
EXOB 9:1 ENDOB	100	26.0±2.4 ab	56.0±6.8 b
EXOB 9:1 ENDOB	1000	24.0±2.4 ab	54.0±6.0 b

[a]Percents within a column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$.

Lab. Bioassay 2C. All combinations of the 9:1 blend elicited significant responses compared to the control (Table 2). Females responded best to the (+):(+) combination. Males responded equally well to the (+):(+) and (±):(+) blends. The racemic:racemic combination elicited low numerical responses by beetles in both sexes.

Lab. Bioassay 2D. (-)EXOB was not attractive by itself (Table 3). When (-)EXOB was combined with the 9:1 blend of (+)EXOB:(+)ENDOB it did not affect the response of female *D. confusus*, but decreased the response of males.

Field experiment 2B. As in the laboratory bioassays, the 9:1 blend of (+)EXOB:(+)ENDOB was the most attractive for *D. confusus* of both sexes in the field (Fig. 5). The response to the 1:1 mixture was lower than to (±)EXOB. In agreement with the previous field experiment (using racemic blends) (Fig. 3), *D. affaber* responded best to the 1:1 mixture of (+) enantiomers (Fig. 5).

Field Exp. 2C. The responses of male and female *D. confusus* to chiral combinations of the 9:1 EXOB:ENDOB blend demonstrated that the (+) enantiomers were responsible for attraction (Fig. 6). Two hypotheses could account for the reduction of catches to the combination of (±)EXOB and (+)ENDOB. One is that the release rate of the active

TABLE 2. Response by *Dryocoetes confusus* in laboratory bioassay 2C to 1 picogram stimuli of combinations of (±) and (+) enantiomers of *exo*-brevicomin (EXOB) and *endo*-brevicomin (ENDOB) in 9:1 blends of EXOB:ENDOB.

	PERCENT RESPONSE ($\bar{X}$±SE) [a]	
STIMULUS	MALES	FEMALES
PENTANE (10 μL)	6.0±4.0 c	6.0±2.4 c
(+)EXOB:(+)ENDOB	42.0±2.0 a	82.0±7.3 a
(+)EXOB:(±)ENDOB	14.0±2.4 b	30.0±6.3 b
(±)EXOB:(+)ENDOB	36.0±2.4 a	40.0±3.2 b
(±)EXOB:(±)ENDOB	16.0±4.0 b	22.0±3.7 b

[a]Percents within a column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test. $P<0.05$.

TABLE 3. Response by *Dryocoetes confusus* in laboratory bioassay 2D to 9:1 blends of (+)-*exo*-brevicomin (EXOB) and (+)-*endo*-brevicomin (ENDOB), and to (-)EXOB tested alone or combined with the binary blend.

STIMULUS	DOSE (picograms)	PERCENT RESPONSE ($\bar{X}$±SE)[a] MALES	FEMALES
PENTANE (10 μL)	0	4.0±2.4 c	6.0±4.0 b
(-)EXOB	0.1	14.0±4.0 bc	10.0±5.5 b
(+)EXOB:(+)ENDOB	0.1	46.0±4.0 a	54.0±5.1 a
(-)EXOB and (+)EXOB:(+)ENDOB	0.1+0.1	26.0±4.0 b	60.0±4.5 a

[a]Percents within a column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test. $P<0.05$.

Figure 5. Numbers of *Dryocoetes confusus* and *D. affaber* caught in Field Exp. 2B to traps baited with blends of (+)-*exo*-brevicomin and (+)-*endo*-brevicomin in three ratios, 10 replicates, July 4-17, 1991. Bars with the same letter within each species are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test on data transformed by $x'=\log(x+1)$, $P<0.05$. Zero catches to unbaited traps were excluded from statistical analysis. More males than females were flying during this experiment.

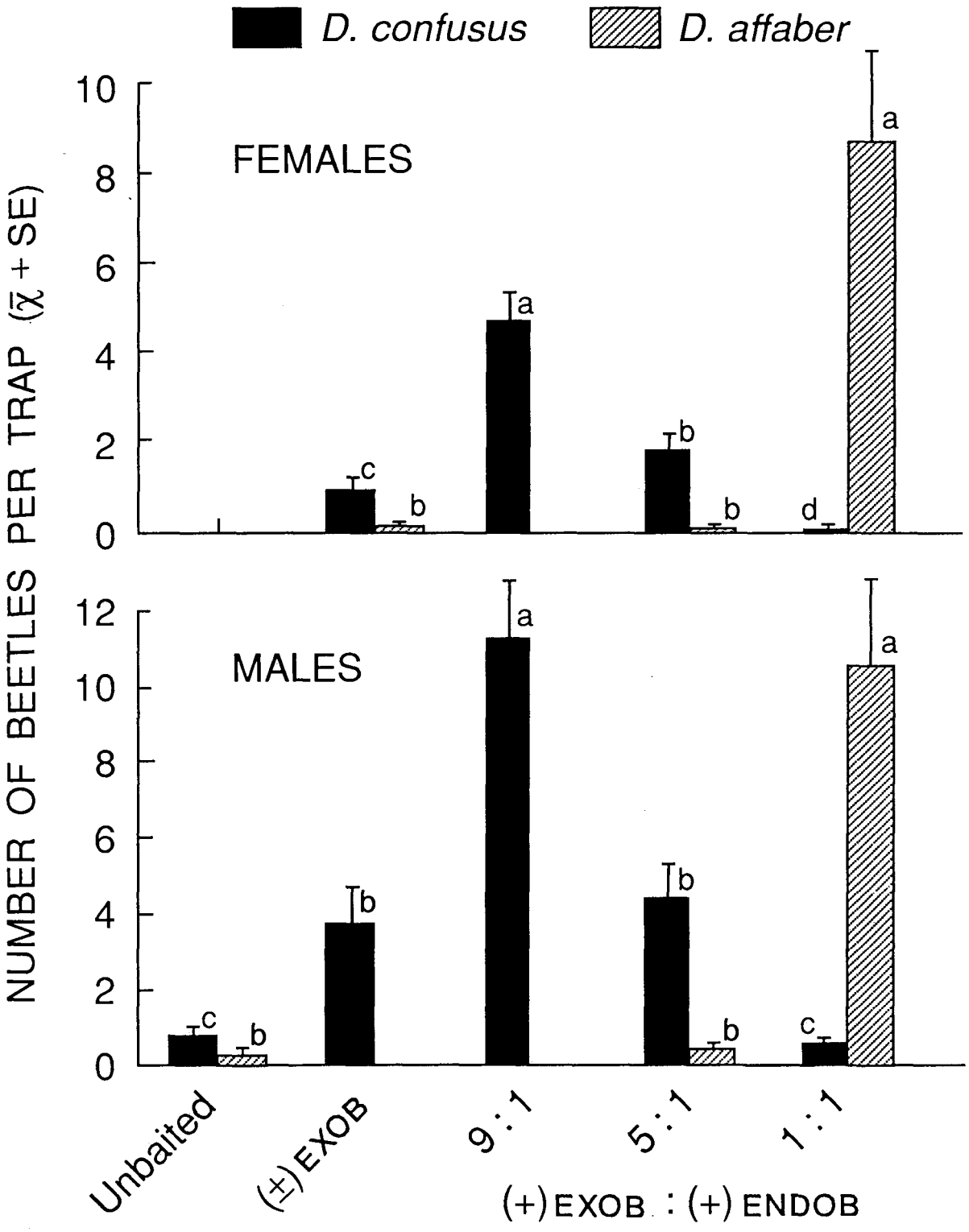
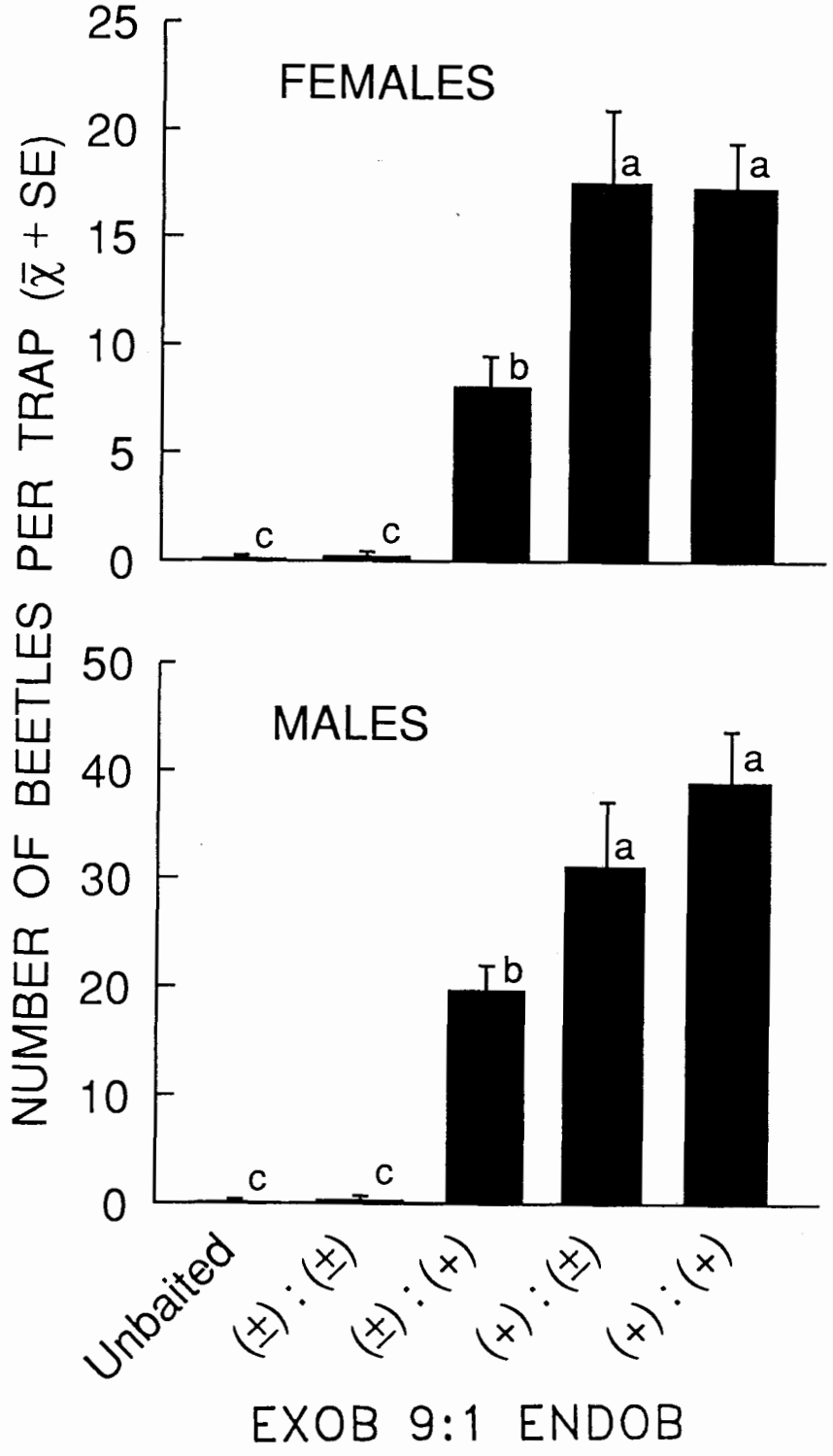

D. confusus
D. affaber
FEMALES
MALES
NUMBER OF BEETLES PER TRAP (x̄ + SE)
Unbaited
(±)EXOB
9:1
5:1
1:1
(+)EXOB : (+)ENDOB

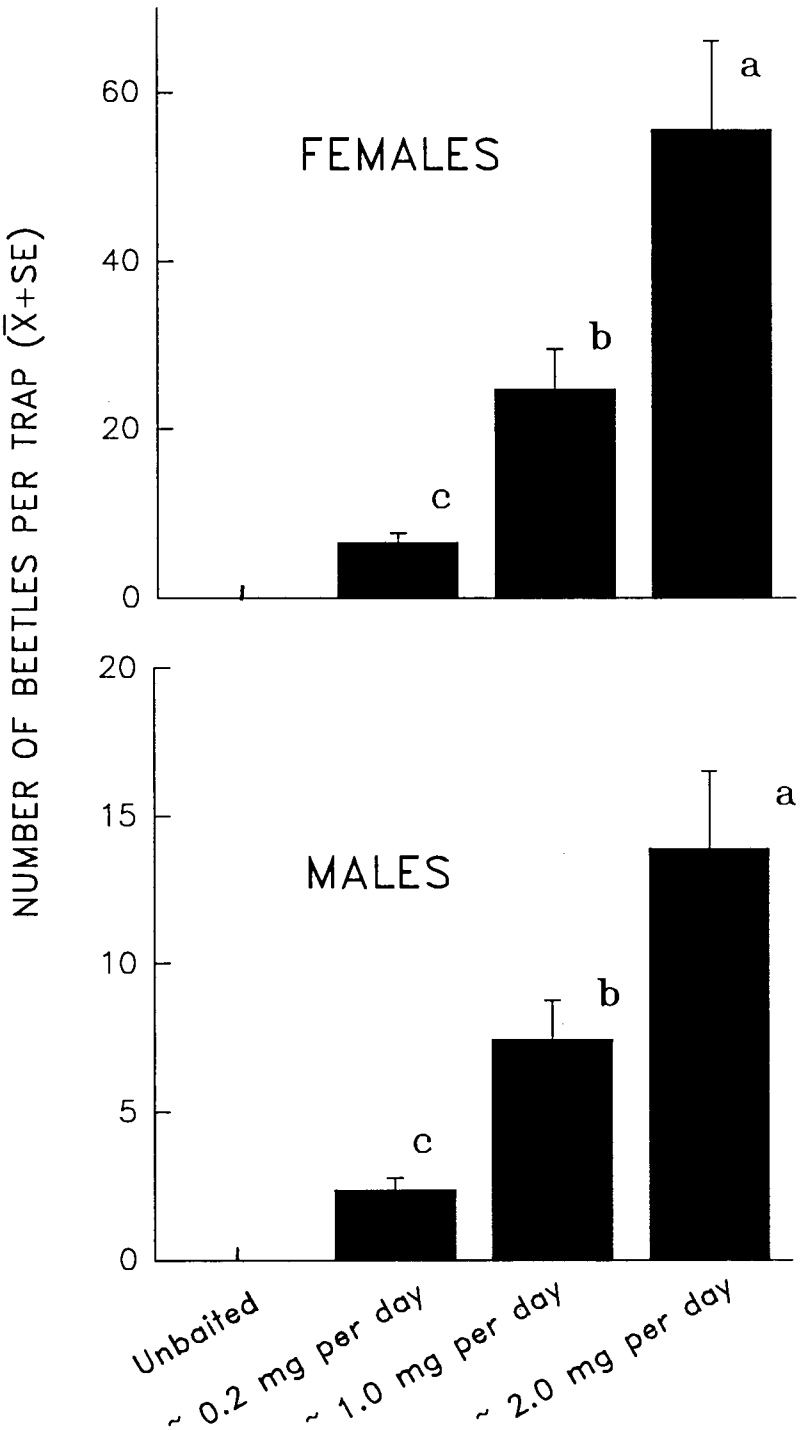
Figure 6. Numbers of *Dryocoetes confusus* caught in Field Exp. 2C to traps baited with chiral combinations of *exo*-brevicomin (EXOB) and *endo*-brevicomin (ENDOB) all in a 9:1 ratio, 20 replicates, July 4-26, 1991. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test on data transformed by x'=log(x+1), $P<0.05$.


FEMALES
MALES
NUMBER OF BEETLES PER TRAP ($\bar{x}$ + SE)
25
20
15
10
5
0
50
40
30
20
10
0
a
b
c
Unbaited
(±) : (±)
(±) : (+)
(+) : (±)
(+) : (+)
EXOB 9:1 ENDOB

(+)EXOB was approximately half the rate achieved when optically active materials were deployed. The other is that the response was partially inhibited by the presence of (-)EXOB. The presence of both (-)EXOB and (-)ENDOB in the (±):(±) combination lowered the catches to a level not significantly different from those to the unbaited controls. This dramatic reduction of catches suggests an inhibitory or blocking effect by the (-) enantiomers when they are combined. A similar reduction in response was observed by Kohnle and Vité (1984) for European *D. autographus*.

Field Exp. 2D. The response of *D. confusus* to the blend (+)EXOB:(+)ENDOB at the 9:1 ratio was dose dependent for both sexes (Fig. 7). This is a confirmation of the multifunctional role of (+)ENDOB for *D. confusus*, since even at relatively high release rates (around 0.2 mg/day of ENDOB), it remains atractive when presented in a ratio of 9:1 with (+)EXOB. This result does not support the hypothesis that the total amount of ENDOB in the environment is responsible for antiaggregation (Stock et al. 1990). The decline in production of EXOB in mated males and the change of the ratio of EXOB:ENDOB concurrently with an undisclosed antiaggregation pheromone produced by mated females (Stock and Borden 1983) may account for antiaggregation effects.

Figure 7. Numbers of *Dryocoetes confusus* caught in field Exp. 2D to traps baited with 9:1 blends of (+)-*exo*-brevicomin and (+)-*endo*-brevicomin released at three different rates, 8 replicates, 5 to 20 August, 1992. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test on data transformed by $x'=\log(x+1)$, $P<0.05$.


NUMBER OF BEETLES PER TRAP (X̄+SE)
FEMALES
60
40
20
0
a
b
c
MALES
20
15
10
5
0
a
b
c
Unbaited
~ 0.2 mg per day
~ 1.0 mg per day
~ 2.0 mg per day

Field Exp. 2E. Despite low catches due to cold weather near the end of the flight period in 1992, the results of this experiment again indicated that for *D. confusus*, the presence of (-)EXOB inhibited the response of males to the attractive (+):(+) combination; females were not affected (Table 4). This result is consistent with those observed in Lab. bioassay 2D (Table 3), and Field Exp. 2A (Fig. 3), in which males responded so poorly to (±):(±) blends that they failed to discriminate among ratios of EXOB:ENDOB. Although I used a 9:1 EXOB:ENDOB blend, the chiral combination (-):(+) was not attractive for *D. confusus*. This combination elicited a response from *D. affaber*, which suggests that the main pheromonal component for *D. affaber* is (+)ENDOB, while high proportions of (+)EXOB inhibit the response in this species.

My results do not agree with those of Stock et al. (1993) who found that the unnatural (-)EXOB enhanced the attraction of *D. confusus* to (+)EXOB. The inhibition of response to the 9:1 blend of (+)EXOB:(+)ENDOB caused by (-)EXOB (Table 4) suggests that this compound could play a role as an antiaggregant in a similar way to that reported for (-)-*trans*-verbenol in the western pine beetle, *Dendroctonus brevicomis* LeConte (Byers 1983).

TABLE 4. Numbers of *Dryocoetes confusus* and *D. affaber* caught in Field Exp. 2E to traps baited with two chiral combinations of blends of *exo*-brevicomin (EXOB) and *endo*-brevicomin (ENDOB) at a 9:1 ratio in a single release device, and with (-)-*exo*-brevicomin (in a separate release device) together with the (+):(+) blend. Aproximate release rate 0.2 mg/day/device. Ten replicates 16 July to 20 August, and six replicates 20 August to 2 October, 1992.

	NUMBER OF BEETLES CAPTURED ($\bar{X}$ ± S.E.)[a]			
	D. confusus		*D. affaber*	
STIMULUS	MALES	FEMALES	MALES	FEMALES
UNBAITED	0	0	0	0
(+)EXOB:(+)ENDOB	1.9±0.2 a	2.2±0.4 a	0	0
(-)EXOB:(+)ENDOB	0.2±0.1 b	0.1±0.1 b	4.0±1.2	4.4±1.2
(+)EXOB:(+)ENDOB with (-)EXOB	0.6±0.2 b	1.9±0.4 a	0	0

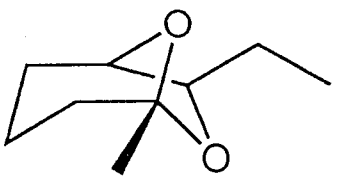
[a]Means within a column followed by the same letter are not significantly different, Friedman test, $P<0.05$. Zero catches in unbaited traps excluded from statistical analysis.

Physiological and Ecological Implications.

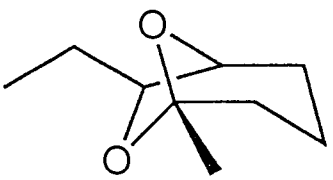
These results demonstrate that a combined effect of geometrical and optical isomers of brevicomin (Fig. 8), determines species specificity and level of response to semiochemicals in two *Dryocoetes* spp.

Specifically, they elucidate three principal phenomena. 1) The ratio of geometrical isomers of EXOB and ENDOB, either as racemic compounds or as (+) enantiomers, determines both the level of response and the species specificity of the blend. 2) As disclosed through varying the ratio of EXOB to ENDOB, both compounds are multifunctional. EXOB can act with ENDOB in a 9:1 ratio as an aggregation pheromone for *D. confusus*, and apparently serves as a synomone which deters *D. affaber* from responding to the ENDOB produced by *D. confusus*. ENDOB, on the other hand, acts at comparatively low levels in combination with EXOB as an aggregation pheromone for *D. confusus*, and at higher levels as an antiaggregation pheromone (Stock et al. 1990). As the relative amount of (+)ENDOB in the blend increases, it also functions as an attractant for *D. affaber* (its hypothesized role as a pheromone is discussed in Section 4). 3) The chiral nature of the pheromones is also of major importance. Optimal response by *D. confusus* to the "correct" 9:1 ratio of geometrical isomers occurs only when the (+) enantiomers

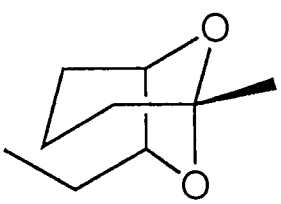
Figure 2. Geometrical and optical isomers of brevicomin (7-ethyl-6,8-dioxabicyclo [3.2.1] octane). 1) (+)-*exo*-brevicomin; 2) (-)-*exo*-brevicomin; 3) (+)-*endo*-brevicomin; 4) (-)-*endo*-brevicomin.



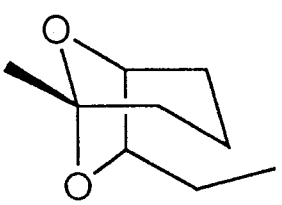
1



2



3



4

of both pheromones are present. Even when high proportions of the "right" geometrical isomer are present, the effect of the "wrong" enantiomer can result in loss of attractiveness and specificity of the chemical message.

The use of multicomponent pheromone blends is a widespread mechanism that provides uniqueness of chemical signals in insects (Silverstein 1981; Cardé 1986; Linn and Roelofs 1989). When one considers the possible combinations resulting from two or more chemicals of varying chirality, mixed in different ratios of geometrical isomers, it appears that insects with such types of pheromones have virtually unlimited means for partitioning of pheromone communication channels. There may be some tolerance to changes in component ratios (Schlyter 1987; Byers 1988). However, overlapping of chemical signals between species can be avoided by very small shifts in these ratios (Figs. 3,5). Optimal responses within a species can be mediated by even smaller shifts (Fig. 4).

2.3 RESPONSE OF *D. CONFUSUS*, TO HOST TREES BAITED WITH ENANTIOSPECIFIC BLENDS OF *EXO*- AND *ENDO*-BREVICOMIN

2.3.1 Introduction

Western balsam bark beetle males produce (+)EXOB (Schurig et al. 1983). Laboratory and field experiments indicated that (±) or (+)-EXOB were attractive to *D. confusus* and (±)EXOB readily induced attack on baited subalpine firs, suggesting that it could be used for semiochemical-based management of *D. confusus* populations (Borden et al. 1987; Stock 1991; Stock et al. 1993). However, the evidence presented in Section 2.2, demonstrated that under laboratory conditions (Fig. 4) and using baited traps in the field (Fig. 5), blends of (+)EXOB and (+)ENDOB at the 9:1 ratio, are more attractive than (±)EXOB.

With the prospect of developing a highly effective tree bait, my objective was to test the hypothesis that enantiospecific blends of EXOB and ENDOB at a 9:1 ratio are better tree baits than (±)EXOB alone.

2.3.2 Materials and Methods

Synthetic Pheromones. Optically pure compounds were synthesized by E.K. Czyzewska (Department. of Chemistry, Simon Fraser University, Burnaby, B.C.) using the Sharpless asymmetric dihydroxilation (Sharpless et al. 1991). I used (+)EXOB (98.1 and 93.1 % chemically and optically pure, respectively), and (+)ENDOB (98.8 and 90.2 % chemically and optically pure, respectively). In order to obtain (±)EXOB free of ENDOB, (+) and (-)EXOB were prepared separately, then mixed, resulting in 95.7 % pure (±)EXOB; no ENDOB was detected by gas chromatographic analysis (Hewlett Packard 5830A and 5880A equipped with flame ionization detectors); 4.3 % were other impurities. I obtained (±)ENDOB (96.4 % pure) from Phero Tech Inc., Delta, B.C.

Field Exp. 2F. A 10-replicate experiment in a randomized complete block design was set up on 25 May, 1992, in a heavily infested mature stand of subalpine fir and Engelmann spruce, located approximately 40 Km West of Merritt, B.C., elevation 1580 m. There were four treatments: (±)EXOB; two blends of EXOB:ENDOB, (+):(±) and (+):(+), both with 9:1 ratios of (+) enantiomers; and an unbaited control. The semiochemical baits were released at approximately 0.2 mg/24 h (Stock 1991), from glass capillary

tubes (1.0 mm I.D.), sealed at one end, placed in open polypropylene tubes, and affixed approximately 1.9 m high in waterproofed cardboard holders (Phero Tech Inc., Delta, B.C.) on the north side of the bole. Experimental and control trees were healthy subalpine firs >20 cm diameter at breast height (dbh) (breast height=1.3 m). They were spaced 33-66 m apart, and were located at least 10 m away from the nearest infested trees.

On 15 September, 1992, beetle attack was assessed on baited and control trees and all surrounding trees >12 cm dbh within 10 m. Trees were classified as unattacked, lightly attacked (characteristically with resin flow on the bole indicating unsuccessful attacks), or mass attacked (with granular frass on the bole or at the base of the tree as evidence of successful attack). Two samples of bark (20 by 20 cm) were taken at approximately 1.9 m high from the East and West sides of the bole of baited trees. The numbers of attack holes and gallery systems with and without larvae were counted in these samples.

Statistical Analysis. I used two-way analysis of variance and the Ryan-Einot-Gabriel-Welsch multiple F test (Schlotzhauer and Littell 1987), on untransformed data for number of attacks per m^2 on baited trees, and on the data transformed by $p'=\arcsin \sqrt{p}$ (Zar 1984) for pooled percents

of surrounding trees. Percent values of 0% were entered as 1/4n and 100% values were entered as 1-1/4n to improve the transformation (Bartlett 1937). In all cases α=0.05 . All analyses were performed with SAS computer software (SAS Institute 1990).

2.3.3 Results and Discussion

All the bait treatments induced similar high levels of mass attack, but there were differences in attack density on baited trees: the (+):(+) EXOB:ENDOB blend elicited the highest attack density (Table 5). The (+):(±) blend caused an intermediate attack density, and (±)EXOB elicited the lowest. A similar trend was observed in the numbers of galleries containing larvae. *Dryocoetes confusus* did not colonize unbaited control trees.

Of 107 trees within 10 m of unbaited trees, only 4.7 % were lightly attacked (Fig. 9). The proportions of all attacked trees and those that were mass attacked increased progressively around trees baited with (±)EXOB, the (+):(±) blend and the (+):(+) blend (Fig. 9). The total numbers of surrounding mass attacked trees per treatment were: unbaited, zero; (±)EXOB, 14; (+):(±) blend, 24; and (+):(+) blend, 39.

TABLE 5. Field Exp. 2F, attack by *Dryocoetes confusus* on subalpine firs baited with (±)-*exo*-brevicomin and 9:1 blends of (+):(±) and (+):(+) *exo*- and *endo*- brevicomin (EXOB and ENDOB). Prospect Road, Merritt Forest District B.C., 25 May to 15 September, 1992.

Bait	Number of replicates	Percent of trees mass attacked	No. of attacks per m^2 on mass attacked trees ($\bar{X}$±SE)[a] Total attacks	Attacks with larvae
Unbaited	10	0.0	0.0 c	0.0 c
(±)EXOB	10	80.0	129.7±10.0 b	81.2±12.0 b
(+)EXOB:(±)ENDOB 9:1	10	80.0	157.8±20.0 ab	106.2±25.9 ab
(+)EXOB:(+)ENDOB 9:1	9	88.9	204.7±18.1 a	160.9±23.6 a

[a]Numbers within a column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$.

Figure 9. Field Exp. 2F. Total attack and mass attack by *Dryocoetes confusus* on subalpine firs within 10 m of trees baited with (±)-*exo*-brevicomin (EXOB) and 9:1 blends of (+):(±) and (+):(+) *exo*- and *endo*-brevicomin (ENDOB). Bars topped by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test on arcsin transformed percents, $P < 0.05$.

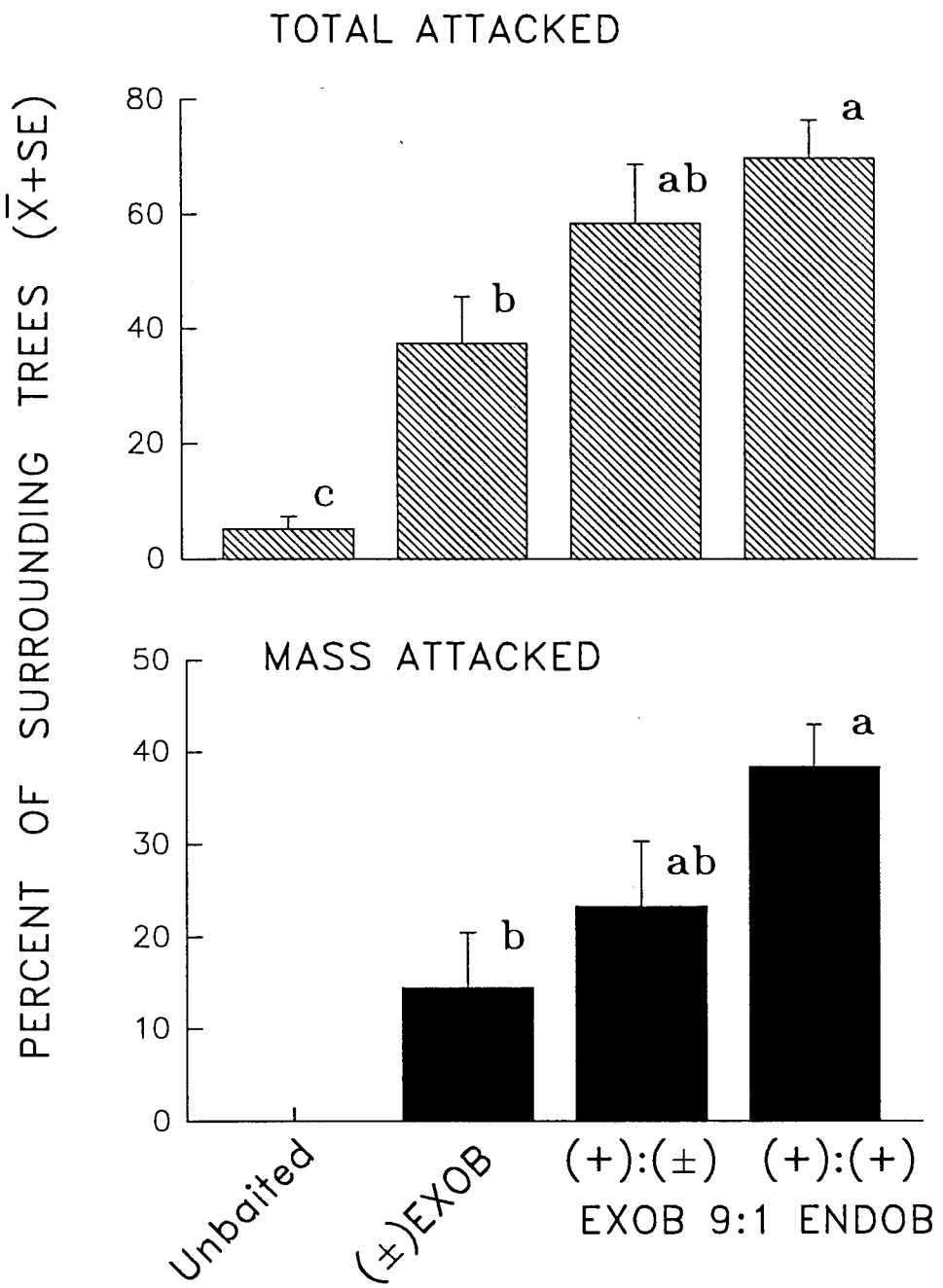

TOTAL ATTACKED
PERCENT OF SURROUNDING TREES (X̄+SE)
80
60
40
20
0
a
ab
b
c
MASS ATTACKED
50
40
30
20
10
0
a
ab
b
Unbaited
(±)EXOB
(+):(±)
(+):(+)
EXOB 9:1 ENDOB

Figure 9

The acute sensitivity of bark beetles to semiochemicals (Payne 1979), makes chemical purity a critical issue in pheromone research. Because of the multifunctionality of ENDOB for *D. confusus* (Stock et al. 1990; Section 2.2), I used "uncontaminated" (±)EXOB. Previous reports using (±)EXOB with *D. confusus*, do not mention the ENDOB content (Borden et al. 1987; Stock 1991; Stock et al. 1990, 1993), and it is possible that small amounts of ENDOB in these studies, contributed to the bioactivity of the EXOB baits.

My results with baited trees support those from laboratory bioassays and trapping experiments presented in Section 2.2, indicating that a 9:1 blend of (+)EXOB:(+)ENDOB is a highly attractive pheromone for *D. confusus*. They also indicate that the synergistic effect of ENDOB is best in the absence of the (-) enantiomer.

(±)EXOB baits are cheaper than those containing optically active materials. However, the superior results with the (+):(+) blend (Table 5, Fig. 9), may justify a greater cost of production. Moreover, the binary blend could be released at lower rates so that the cost per bait becomes competitive.

Promising results were obtained by Stock et al. (1993), using (±)EXOB for containment and concentration of *D. confusus* populations. I contend that the use of a 9:1 blend of (+)EXOB:(+)ENDOB would improve the efficacy of this tactic and could lead to its operational implementation.

3. HOST COMPOUNDS AS KAIROMONES FOR *DRYOCOETES CONFUSUS*

3.1 INTRODUCTION

Phytophagous insects have sensory receptors that enable them to perceive stimuli associated with host and non-host plants. These stimuli can be visual, mechanical, gustatory, or olfactory (Visser 1986). Whether monophagous or polyphagous, insects must select a suitable plant species and then proceed to the most suitable individual host (Raffa 1988).

Host selection by bark beetles involves: orientation, landing, probing, biting, feeding, gallery construction, and oviposition (Raffa and Berryman 1982; Visser 1986). In some species it appears that flying beetles land in response to visual cues, arriving at random on host and non host trees (Moeck et al. 1981). Landing beetles would then bite the plant tissues under the influence of excitants and continue feeding in the absence of deterrents (Doskotch et al. 1970); pheromone release would start and mass attraction would take place. If the stimuli were found to be unsuitable, the

beetle would resume flight and reinitiate the process (Raffa 1988).

On the other hand, host tree volatiles elicit a long-range kairomonal response from several bark beetle species (Chapman 1962; Vité and Gara 1962; Peacock et al. 1971, 1984; Werner 1972; Moeck 1978; Volz 1988; Miller et al. 1986; Miller and Borden 1990b). Two species, *Tomicus piniperda* (Byers et al. 1985) and the native elm bark beetle, *Hylurgopinus rufipes* (Eichhoff) (Swedenborg et al. 1988), appear to rely on host volatiles alone to select their hosts, without supplementing host kairomones with aggregation pheromones. In a computer-based simulation of natural host selection, Gries et al. (1989) showed that without primary attraction (presumably based on attractive host kairomones), selection of hosts by *Ips typographus* L. would be too inefficient to sustain a population.

In general, during orientation and host selection by pioneer bark beetles of many species, there is an integration of visual stimuli and chemical signals produced by the host. These signals provide primary information about the species and physiological state of the tree. After initial attack by pioneer beetles, secondary attraction occurs in which pheromones interact with host odorants to elicit a massive response from dispersing conspecific beetles (Borden

at al. 1975, 1986; Borden 1982; Lanier 1983; Payne and Coulson 1985).

There is evidence of response to host-related stimuli in several *Dryocoetes* spp. *Dryocoetes affaber* and *D. autographus* were attracted to uninfested spruce logs (Furniss et al. 1976). For *D. autographus*, this attraction may be enhanced by the presence of ethanol used as a tree bait (Souto 1974), or released from traps in combination with monoterpenes (Chénier and Philogène 1989). *D. confusus* shows a preference for windfalls, freshly felled trees, and diseased trees (Stock 1991; Erickson and Ferris 1992). In field trapping experiments, *D. confusus* were attracted to uninfested logs, and in laboratory bioassays males (and to a lesser extent females) responded to host volatiles trapped in Porapak-Q (Stock and Borden 1983). However, Borden et al. (1987) found no statistical difference in the response of *D. confusus* to traps baited with (±)EXOB alone or in combination with one or all of the six major monoterpenes present in *A. lasiocarpa* [α-pinene, ß-pinene, (+)-3-carene, (-)-ß-phellandrene, myrcene, and limonene] (Zavarin et al. 1971).

Clearly, there was a need for a systematic approach (Silverstein et al. 1967), to the identification of host kairomones for *D. confusus*. Therefore, my objective was to isolate and identify attractive host volatiles that may act alone or in combination with pheromones to mediate the colonization behavior of *D. confusus* in nature.

3.2 MATERIALS AND METHODS

Collection of Insects and Hosts. Logs of subalpine fir, both uninfested and infested with *D. confusus*, were obtained from trees felled near Merritt, B.C. The infested logs were placed in screened cages in the laboratory; emerging adults were sexed and kept on moistened paper at 5^{o}C until used for laboratory bioassays.

Collection, Fractionation, and Analysis of Host Volatiles. Outer bark, phloem, and sapwood were taken from field collected logs originally from the top, middle and bottom part of uninfested trees. Host materials were macerated and subjected to steam distillation in a 30 cm glass Dufton column. The resulting "phloem" oil (0.835 % of starting material by weight), was analyzed by GC and GC-MS

using Hewlett Packard 5830A and 5880A gas chromatographs equipped with capillary inlet systems, flame ionization detectors and open tubular glass columns (30 m X 0.5 mm I.D.), coated with SP-1000 (Supelco Inc. Bellefonte, PA). The temperature program was 70°C for 2 min, then 4°C/min to 180°C holding for 20 min. Chirality of optically active compounds was determined in a Hewlett Packard 5890 gas chromatograph with a Chirasil-Dex (8) column (25 m X 0.25 mm I.D.) (V. Schürig, University of Tübingen, Germany) at 90°C. Coupled gas chromatography-mass spectrometry (GC-MS) was performed with a Hewlett Packard 5895B GC-MS-DS fitted with a fused silica column (30 m X 0.33 mm I.D.) coated with SP-1000 (J & W Scientific Inc. Folsom, CA).

Fractionation of phloem oil and active fractions by micropreparative GC used 5 μL aliquots of phloem oil per run and was conducted with a Varian 1200 instrument fitted with a stainless steel column (3.05 m X 3.18 mm O.D.), packed with 10% SP-1000 on Supelcoport (100-120 mesh) (Supelco Inc.), a 10:1 effluent splitter, FID, and a thermal gradient collector (Brownlee and Silverstein 1968). The temperature program was 70°C for 2 min, then 4°C/min to 180°C holding for 5 min. Helium was the carrier gas at a flow rate of 40 cm/sec.

Laboratory Bioassays. The response of *D. confusus* to phloem oil, fractions thereof, and synthetic chemicals (Table 6), were assessed using walking beetles in an open arena olfactometer (Wood and Bushing 1963; Stock and Borden 1983). Groups of 10 active beetles (total 50 beetles) of either sex were exposed for 2.5 min to an air stream (700 mL/min) containing volatile stimuli applied in 10 μL of pentane to a filter paper wick. Pentane (10 μL) was used as a control. Room temperature was 20-22^{o}C; room lightning was diffuse and of low intensity (22.57 lux).

I tested the response to 8.5 μg of phloem oil (equivalent to the amount of oil in 1 mg of phloem), and then to 10 μg fractions of phloem oil following the sequence in Fig. 10. In each case, fractions were tested both alone and together with 100 or 200 pg of the attractive pheromone (±)EXOB. Attractive fractions were refractionated until the resulting fractions contained very few compounds, which could be identified by GC-MS. Synthetic host volatiles were tested at a dose of 1 μg; (±)EXOB was tested at doses of 10 pg.

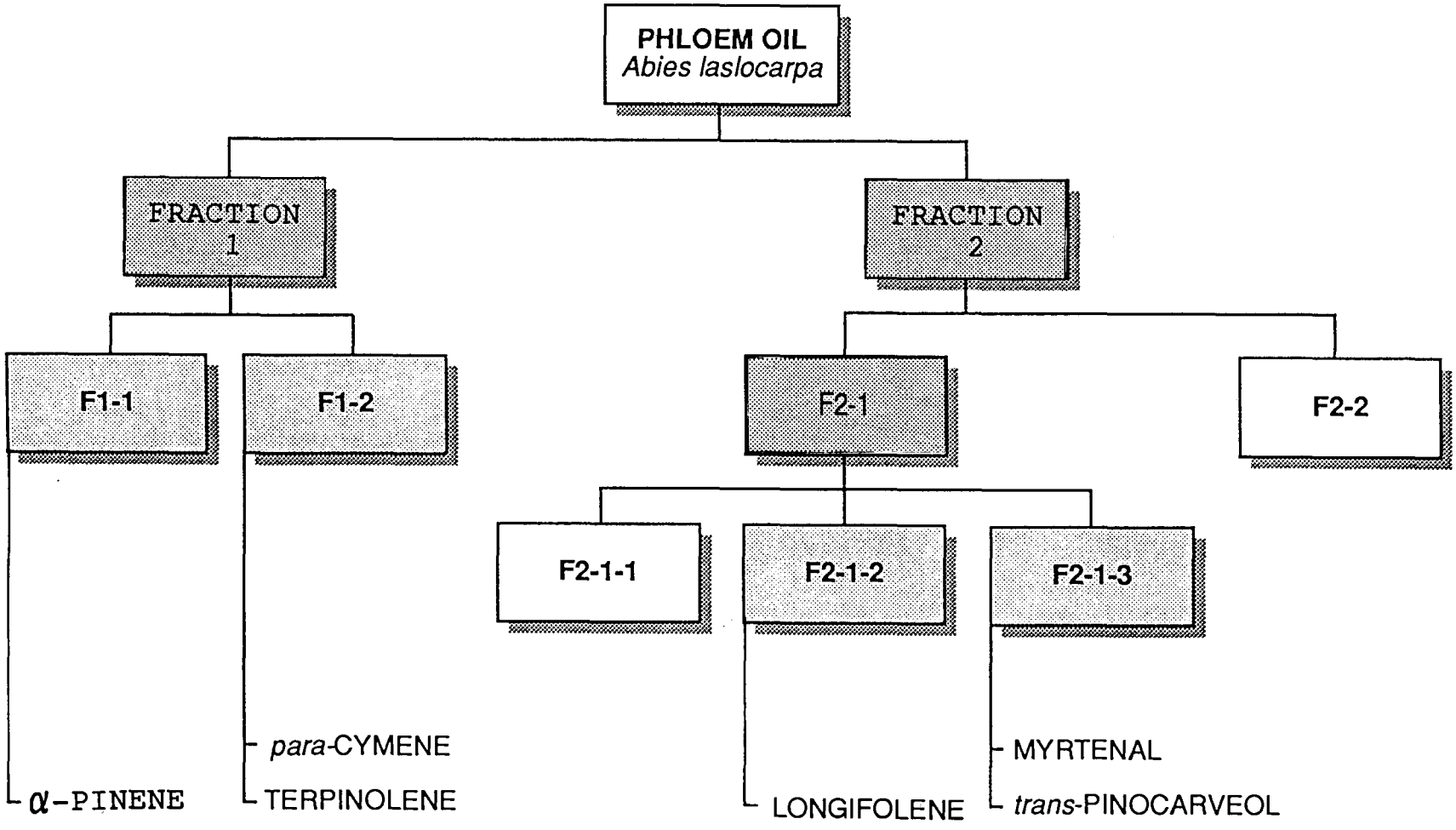
By 1991, research on the pheromonal complex of *D. confusus*, indicated that a 9:1 blend of (+)EXOB:(+)ENDOB, was more attractive than (±)EXOB (Section 2). Therefore, a laboratory bioassay was carried out to evaluate the response

TABLE 6. List of the synthetic chemicals employed in the isolation and identification and field testing of kairomones for *D. confusus*, including specifications and suppliers.

Compound	Chemical purity %	Optical purity %	Supplier	Release rate[b] mg/day
(±)-*exo*-brevicomin	96.3		Phero Tech Inc. Delta, B.C.	0.2
(+)-*exo*-brevicomin	98.1	93.1	B.D. Johnston[a]	0.2
(+)-*endo*-brevicomin	98.8	90.2	"	0.2
(+)-*exo*-brevicomin	98.8	94.0	E.K. Czyzewska[a]	0.2
(1*S*)-(-)-α-pinene			Sigma Chem. Co., St. louis, MO.	12.1
(±)-α-pinene			"	
(+)-longifolene			"	0.4
(1*R*)-(-)-myrtenal	98.4	72.5	H.D. Pierce Jr.[a]	0.9-2.0
terpinolene			Union Camp Co., Jacksonville, FL.	3.1
trans-pinocarveol	95		H.D. Pierce Jr.[a]	2.5
para-cymene			Eastman	3.2

[a]Department of Chemistry, Simon Fraser University, Burnaby, B.C.
[b]Release devices as follows: glass capillary tube (1.0 mm I.D.) sealed at one end, inside an open micro-centrifuge tube (*exo*- and *endo*-brevicomin); glass vials with 1 cm long glass capillary protruding through cap (longifolene, phloem oil); bubble caps (Phero Tech Inc.) with 0.5% A0330 stabilizer (*trans*-pinocarveol); closed 1.5 mL polyethylene micro-centrifuge tubes (Evergreen Scientific, Los Angeles, CA. (*para*-cymene, α-pinene, terpinolene); closed 1.5 mL polypropylene micro centrifuge tube (Quality Plastics, Petaluma, CA) formulated in 10% mineral oil and 1% BHT [*R*-(-)-myrtenal, 1991]; bubble cap with 10% dioctyl adipate and 5% BHT [*R*-(-)-myrtenal, 1992]. All release rates determined by weight loss at 22-26°C in the laboratory.

Figure 10. Sequence of the fractionation of *Abies lasiocarpa* phloem oil, with shadowed boxes indicating fractions attractive to *Dryocoetes confusus* in laboratory bioassays.


PHLOEM OIL
Abies laslocarpa
FRACTION 1
FRACTION 2
F1-1
F1-2
F2-1
F2-2
F2-1-1
F2-1-2
F2-1-3
α-PINENE
para-CYMENE
TERPINOLENE
LONGIFOLENE
MYRTENAL
trans-PINOCARVEOL

to the 9:1 blend (0.01 pg) combined with phloem oil (8.7 μg).

Field Experiments. Trapping experiments were conducted in a subalpine forest located aproximately 40 km west of Merritt, B.C. (altitude 1450-1600 m). Twelve-unit, multiple-funnel traps (Lindgren 1983; Phero Tech Inc. Delta, B.C.) were placed 15 m apart in randomized complete blocks, with 8 to 10 replicates. All field experiments employed the 9:1 blend of (+)EXOB: (+)ENDOB (or "9:1 blend"). Release devices and rates for all materials are given in Table 6.

Field Exp. 3A. In 1991 four host volatiles that appeared to be attractive in the laboratory were evaluated. Terpinolene, *p*-cymene, myrtenal, and *trans*-pinocarveol were tested individually or together with the 9:1 blend. The pheromones and unbaited traps were also included as control treatments.

Field Exp. 3B. In 1992, I tested two new candidates. (+)-Longifolene and (-)-α-pinene (formulated at the natural 74% optical purity),were tested alone or in combination with the 9:1 blend; unbaited traps were used as controls.

Field Exp. 3C. In 1993. I evaluated 9:1 blend alone and in combination with either or both (-)-α-pinene, and

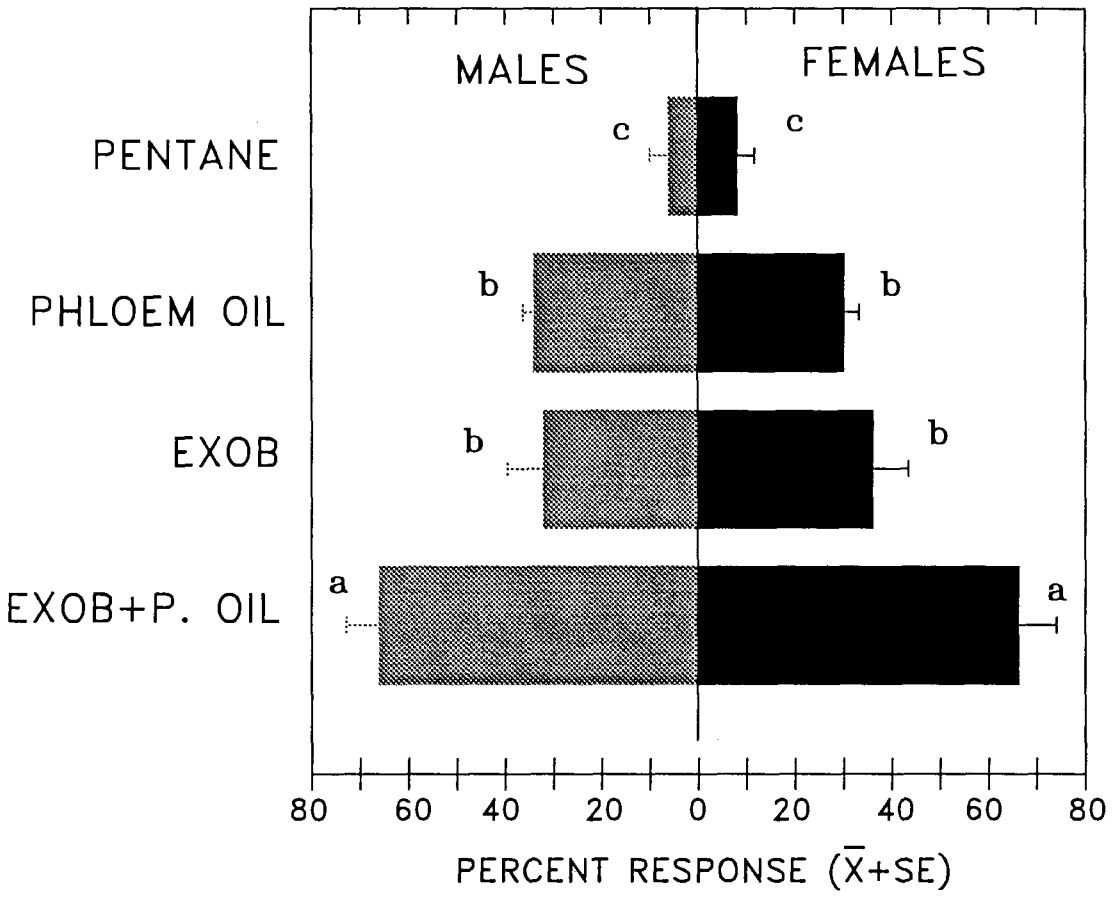
myrtenal, the most promising candidates in previous experiments.

Statistical Analysis. Laboratory bioassay results were analized by one-way analysis of variance (ANOVA) and the Ryan-Einot-Gabriel-Welsch multiple F or "REGWF" test (Schlotzhauer and Littell 1987) utilizing percentages of positive responders converted to p'=arcsine $\sqrt{p}$, to approximate a normal distribution (Zar 1984). Percent values of 0 % were recorded as 1/4 n to improve the transformation (Bartlett 1937). For field trapping experiments I used two-way ANOVA and the REGWF test on numbers of beetles captured transformed by x'=log(x+1) to remove heteroscedasticity (Zar 1984). If data were not then normally distributed I used the non-parametric Friedman test (Friedman 1937; Conover 1980). In all cases α=0.05 . I employed SAS computer software (SAS Institute 1990) for the analyses.

3.3 RESULTS AND DISCUSSION

Laboratory Bioassays. *D. confusus* of both sexes responded to phloem oil, to (±)EXOB, and to the two stimuli combined, in an additive rather than synergistic manner (Fig. 11). This result verified those of Stock and Borden (1983), demostrating the existence of attractive host

Figure 11. Response of *Dryocoetes confusus* in a laboratory bioassay to 200 pg stimulus of (±)-*exo*-brevicomin (EXOB), 8.5 μg of *Abies lasiocarpa* phloem oil, and to a combination of both stimuli. Pentane (10 μL) was the control. Percents with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$.


MALES
FEMALES
PENTANE
PHLOEM OIL
EXOB
EXOB+P. OIL
c
c
b
b
b
b
a
a
80
60
40
20
0
20
40
60
80
PERCENT RESPONSE (X̄+SE)

kairomones, and justifying the continuation of an isolation and identification program.

Initial separation of the phloem oil (Fig. 10) yielded two fractions, F1 containing monoterpenes, and F2 containing sesquiterpenes and terpene-alcohols (Fig. 12). Both fractions were attractive to males (but not to females) at levels similar to those for phloem oil in Fig. 11. The combination of (±)EXOB with F2 or F1 and F2 doubled the response. Further separation of F1 yielded F1-1 and F1-2, both of which significantly enhanced attraction of females to the combinations with (±)EXOB. In F2, subfraction F2-1, but not F2-2, was significantly attractive to both sexes alone and in additive fashion when combined with (±)EXOB. Final separation of F2-1 gave an inactive F2-1-1 and active F2-1-2 and F2-1-3 (Fig. 10).

At this point, analysis by GC-MS disclosed the identities of the most conspicuous compounds in the active fractions (Fig. 12). Five monoterpenes that had been found to be inactive by Borden et al. (1987) (myrcene, 3-carene, ß-pinene, ß-phellandrene, and limonene), were not considered further. Six compounds, (-)-α-pinene, *para*-cymene, terpinolene, (+)-longifolene, (1*R*)-(-)-myrtenal, and *trans*-pinocarveol (Fig 12) were attractive alone or in combination with (±)EXOB in laboratory bioassays (Table 7).

Figure 12. Gas-liquid chromatogram of *Abies lasiocarpa* phloem oil indicating the approximate points of fractionation, and the peaks corresponding to the isolated compounds tested in laboratory bioassays. Peaks numbered 1-5 designate ß-pinene, myrcene, (+)-3-carene, limonene, and (-)-ß-phellandrene, all of which were inactive in field tests (Borden et al. 1987) and were not considered further.

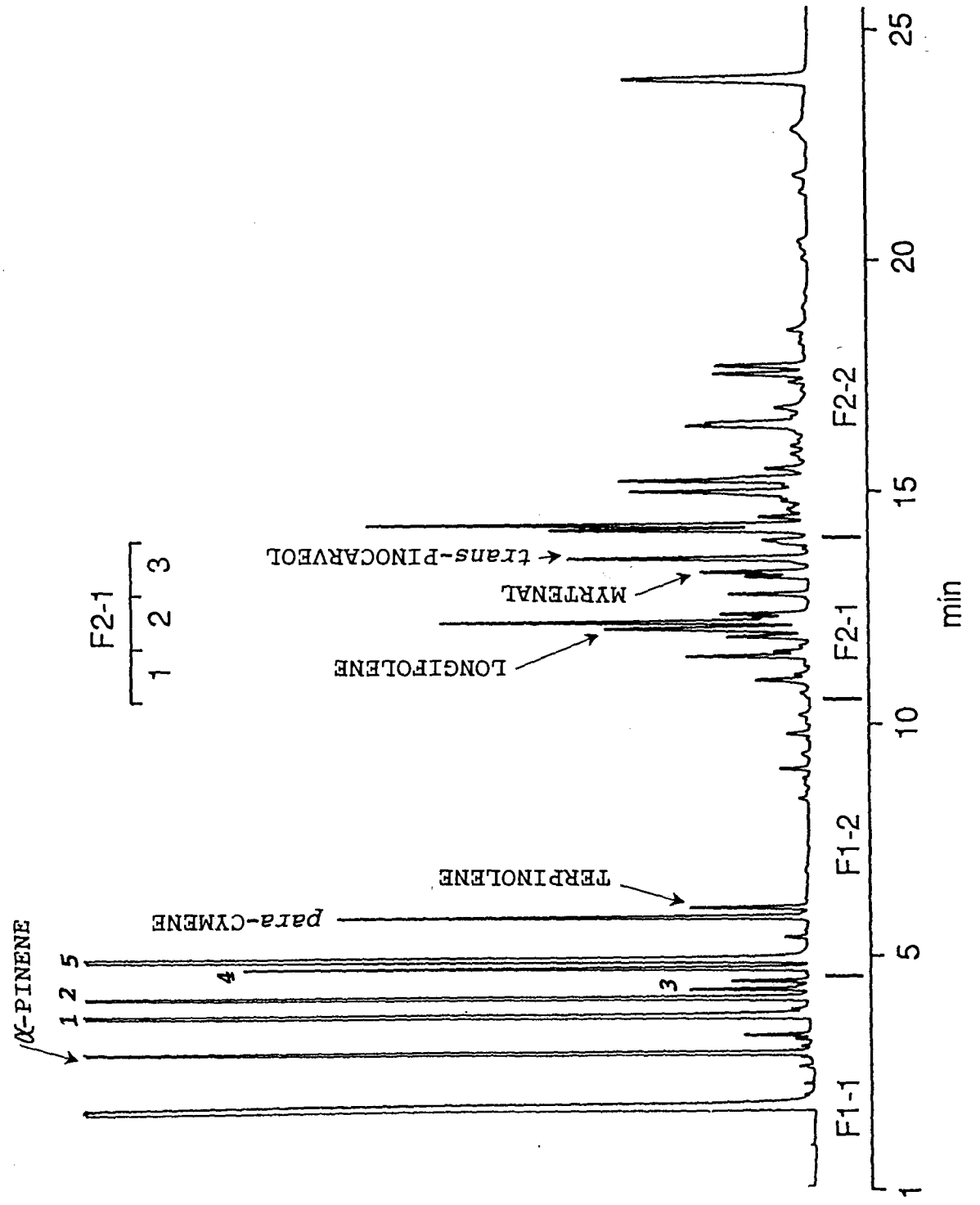

α-PINENE
1 2 5
4
3
para-CYMENE
TERPINOLENE
LONGIFOLENE
MYRTENAL
trans-PINOCARVEOL
F2-1
1 2 3
F1-1
F1-2
F2-1
F2-2
1
5
10
15
20
25
min

TABLE 7. Response by *Dryocoetes confusus* in laboratory bioasssays to candidate host kairomones alone, or in combination with (±)EXOB. Doses were 10 pg for (±)EXOB and 1 μg for candidate compounds in all cases. Fifty beetles of each sex tested for each treatment

CANDIDATE COMPOUND	TREATMENT	PERCENT RESPONSE ($\bar{X}$±SE)[a] MALES	FEMALES
(-)-α-PINENE (αP)	PENTANE	8.0±2.0 c	4.0±2.4 c
	(±)EXOB	20.0±4.5 b	26.0±5.1 b
	αP	24.0±2.4 ab	26.0±2.4 b
	αP + (±)EXOB	38.0±4.9 a	48.0±5.8 a
para-CYMENE (*p*C)	PENTANE	8.0±3.7 b	10.0±3.2 c
	(±)EXOB	22.0±3.7 ab	26.0±6.0 b
	*p*C	12.0±3.7 b	18.0±3.7 bc
	*p*C + (±)EXOB	34.0±5.1 a	52.0±3.7 a
TERPINOLENE (TERP)	PENTANE	6.0±2.4 b	4.0±2.4 b
	(±)EXOB	22.0±3.7 a	20.0±3.2 a
	TERP	22.0±4.9 a	24.0±4.0 a
	TERP + (±)EXOB	30.0±5.8 a	30.0±3.2 a
(+)-LONGIFOLENE (LON)	PENTANE	6.0±2.4 c	4.0±2.4 c
	(±)EXOB	20.0±3.2 b	26.0±4.0 b
	LON	18.0±3.7 b	24.0±5.1 b
	LON + (±)EXOB	42.0±4.9 a	74.0±5.1 a
(*R*)-(-)-MYRTENAL (MY)	PENTANE	6.0±4.0 d	8.0±3.7 c
	(±)EXOB	18.0±2.0 c	24.0±2.4 b
	MY	34.0±5.1 b	30.0±3.2 b
	MY + (±)EXOB	54.0±2.4 a	56.0±2.4 a
trans-PINOCARVEOL (TPC)	PENTANE	6.0±4.0 d	8.0±3.7 c
	(±)EXOB	18.0±2.0 c	24.0±2.4 b
	TPC	26.0±5.1 bc	24.0±4.0 b
	TPC + (±)EXOB	38.0±3.7 ab	46.0±5.1 a

[a]Percents related to a candidate compound, within a column, and followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test. $P<0.05$.

In laboratory bioassay, the combination of phloem oil with the 9:1 blend of (+)EXOB:(+)ENDOB elicited higher response from beetles of both sexes than to the 9:1 blend alone (Fig. 13). The 0.01 pg stimulus of the 9:1 blend was 20 000 times lower than the dose of (±)EXOB required to elicit equivalent levels of response (Fig. 11). The results of this experiment justified the use of the 9:1 blend in field conditions.

Field Exp. 3A. None of the host volatiles tested was attractive by itself (Fig 14). The response of males to the combination of myrtenal and the 9:1 blend was significantly higher than the response to the 9:1 blend alone. A similar numerical trend was observed with females. Myrtenal tends to oxidize rapidly under field conditions; formulation of this compound in solution with mineral oil and an antioxidant (BHT) stabilized this compound for further field testing.

In Field Exp. 3B, the response of both male and female beetles to the 9:1 blend was significantly increased in the presence of (-)-α-pinene (Fig. 15). This result appears to agree with those of Borden et al. (1987), who observed a similar (but not significant) trend with (±)-α-pinene in combination with (±)EXOB. However, it is possible that

Figure 13. Response of *Dryocoetes confusus* in laboratory bioassay to 0.01 pg of a 9:1 blend of (+)EXOB:(+)ENDOB, 8.7 μg of *Abies lasiocarpa* phloem oil, and to a combination of both stimuli. Pentane (10 μL) was the control. Percents with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$.

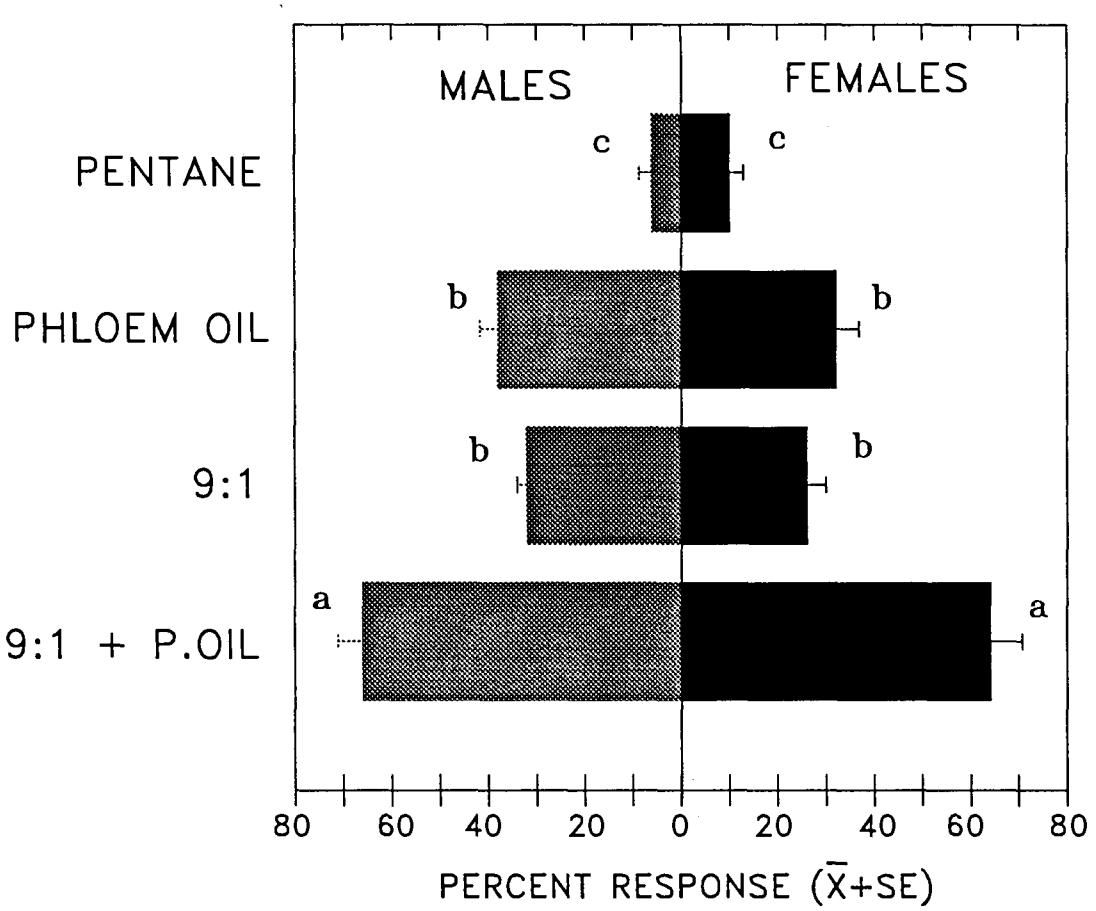

MALES
FEMALES
PENTANE
PHLOEM OIL
9:1
9:1 + P.OIL
c
c
b
b
b
b
a
a
80
60
40
20
0
20
40
60
80
PERCENT RESPONSE (X̄+SE)

Figure 14. Numbers of *Dryocoetes confusus* caught in Field Exp. 3A to traps baited with four individual host compounds, with a 9:1 blend of (+)EXOB:(+)ENDOB; and with combinations of host compounds with the 9:1 blend, 10 replicates 18 July to 8 August, 1991. TPC, *trans*-pinocarveol; TERP, terpinolene. Bars with the same letter are not significantly different, Friedman test, $P<0.05$.

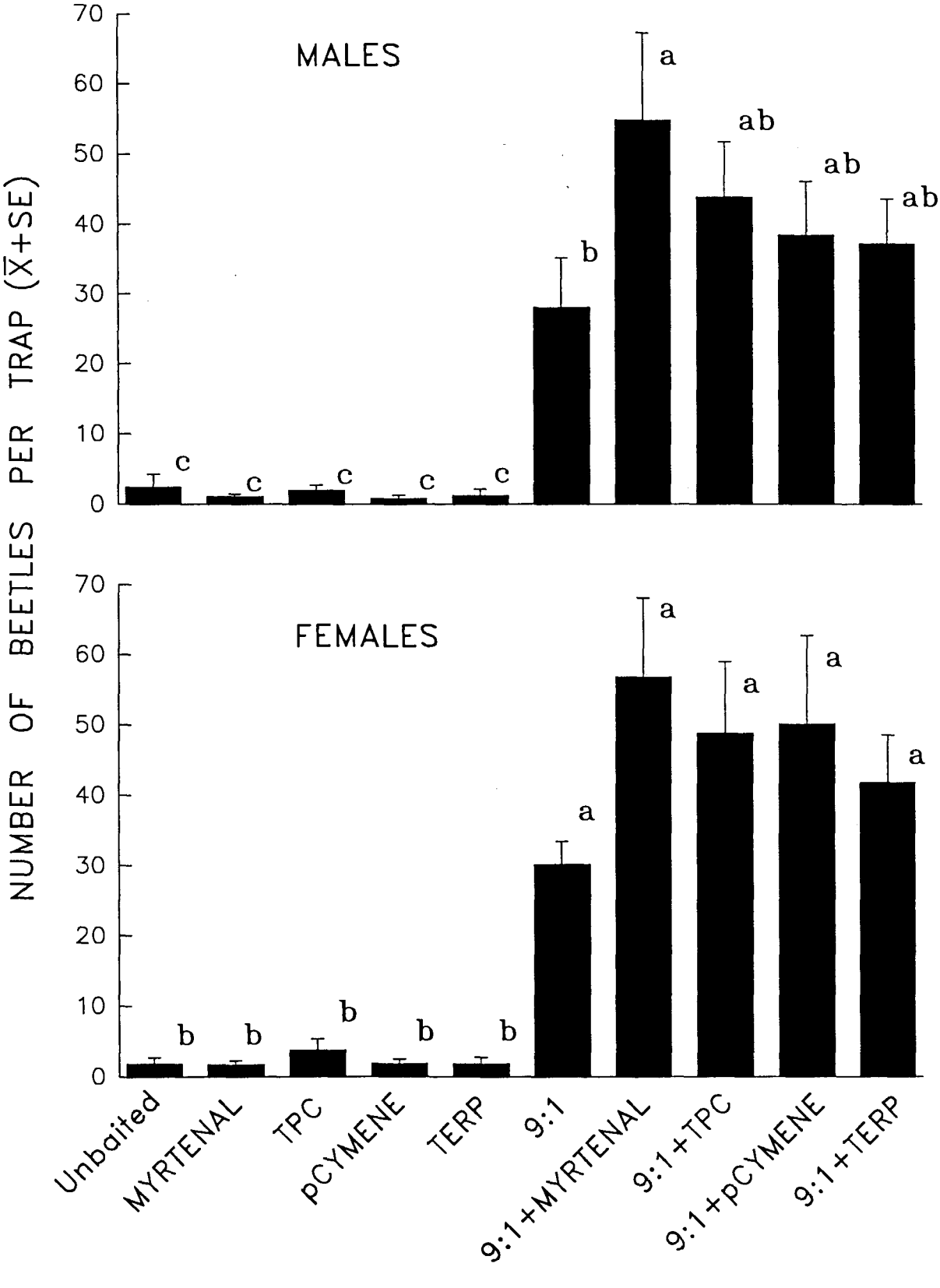
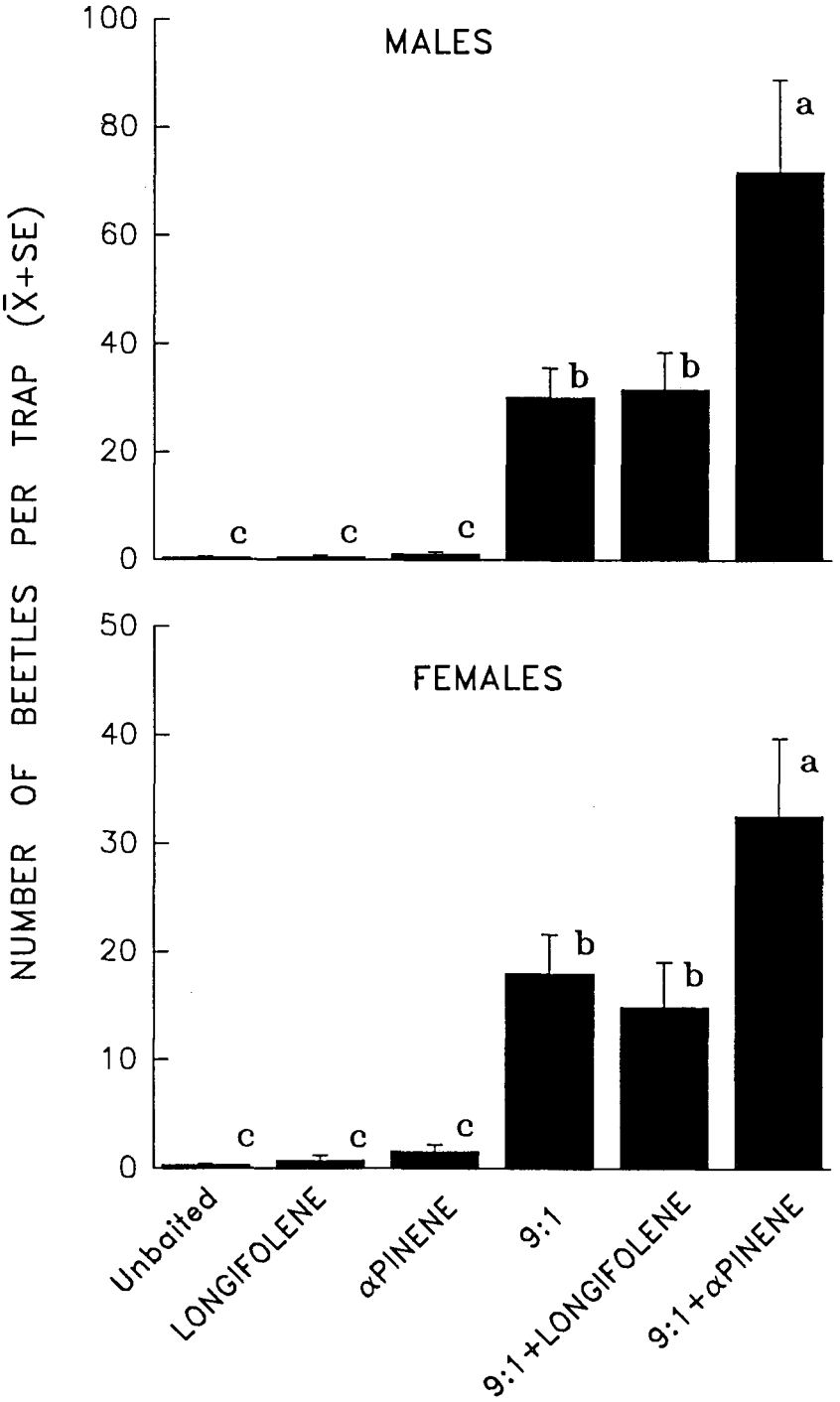

MALES
FEMALES
NUMBER OF BEETLES PER TRAP (X̄+SE)
0
10
20
30
40
50
60
70
c
c
c
c
c
b
a
ab
ab
ab
b
b
b
b
b
a
a
a
a
a
Unbaited
MYRTENAL
TPC
pCYMENE
TERP
9:1
9:1+MYRTENAL
9:1+TPC
9:1+pCYMENE
9:1+TERP

Figure 15. Numbers of *Dryocoetes confusus* caught in Field Exp. 3B to traps baited with two individual host compounds, with a 9:1 blend of (+)EXOB:(+)ENDOB; and with combinations of individual host compounds and the 9:1 blend, 10 replicates 1-29 July, 1992. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test on data transformed by $x'=\log(x+1)$, $P<0.05$.


MALES
FEMALES
NUMBER OF BEETLES PER TRAP (X̄+SE)
100
80
60
40
20
0
50
40
30
20
10
0
a
b
b
c
c
c
Unbaited
LONGIFOLENE
αPINENE
9:1
9:1+LONGIFOLENE
9:1+αPINENE

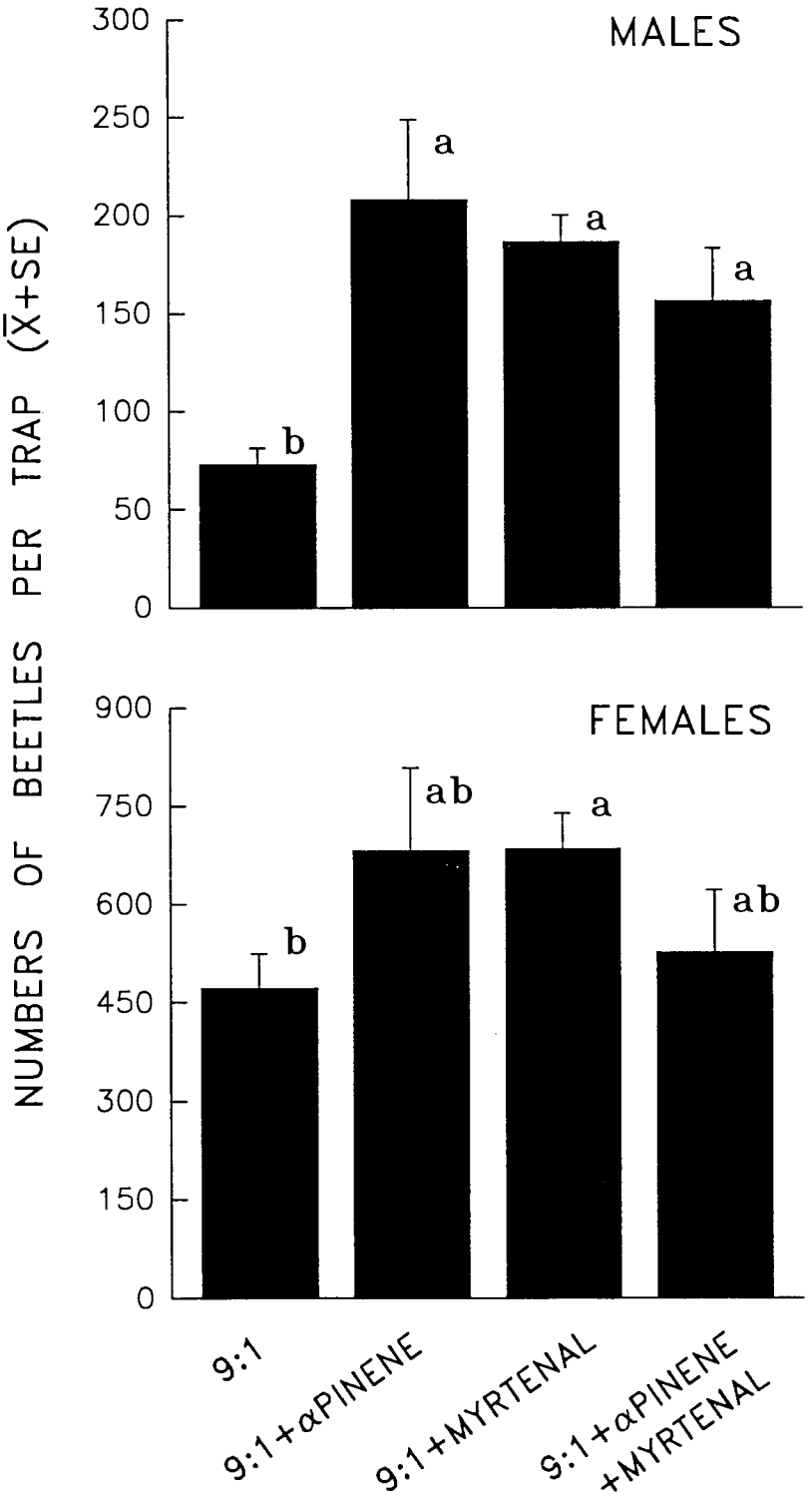
D. confusus responds preferentially to the natural (-) enantiomer. *Dendroctonus valens* showed chiral specificity in the response to α-pinene (Hobson et al. 1993). α-Pinene has been reported as a kairomone for a number of scolytids (Borden 1985), including *Dryocoetes autographus* (Kangas et al. 1965; Chénier and Philogène 1989).

Field Exp. 3C. Beetles of both sexes responded at significantly higher levels to the 9:1 blend with myrtenal than to the pheromones alone (Fig. 16). A similar result occurred with the response of males to the 9:1 blend plus α-pinene but relatively high variability in the data reduced the response by females to the combined bait to near significance (P=0.07). The bubble caps used as a release device for myrtenal in 1993, provided a higher release rate per device (2 mg/day) than the previously used device. They were efficient in protecting myrtenal from polymerization (as determined by GC analysis of used baits), and were also practical for use in the field.

For neither sex was the response enhanced in the presence of both α-pinene and myrtenal over that to combinations involving either host compound (Fig. 16).

This result suggests the existence of generalist type receptor cells keyed to numerous host volatiles, as occurs

Figure 16. Numbers of *Dryocoetes confusus* caught in Field Exp. 3C to traps baited with combinations of individual host compounds, with the 9:1 blend (+)EXOB:(+)ENDOB, and with the triple combination of the 9:1 blend plus the two host compounds, 8 replicates 13 July to 6 August, 1993. Bars with the same letter are not statistically different, Ryan-Einot-Gabriel-Welsch multiple F test on data transformed by $x'=\log(x+1)$, $P<0.05$.


MALES
FEMALES
NUMBERS OF BEETLES PER TRAP (X̄+SE)
300
250
200
150
100
50
0
900
750
600
450
300
150
0
a
a
a
b
ab
a
ab
b
9:1
9:1+αPINENE
9:1+MYRTENAL
9:1+αPINENE +MYRTENAL

in a number of other scolytid species (Mustaparta 1984). Such generalist response would explain the lack of increased attraction in several other experiments involving various combinations of the five candidate kairomones (results not shown).

Few host kairomones have been systematically isolated and identified for bark beetles (Silverstein 1970; Pearce et al. 1975). My research has shown that two host compounds [(-)-α-pinene, and (*R*)-(-)-myrtenal], consistently enhanced attraction by *D. confusus* to species-specific pheromones, and can be considered as kairomones.

The response by *D. confusus* in laboratory bioassays to these and other host compounds (Table 7) suggests that they may be involved in the primary attraction to host logs found by Stock and Borden (1983). The failure to duplicate this response in the field with individual host compounds (Figs. 14, 15), or with various combinations of them (results not shown) suggests that some of them could act only at close range, or that unknown compounds may act in concert with the newly discovered kairomones. Electrophysiological techniques (GC-EAD) (Cork et al. 1990) may be useful to resolve host odours which constitute much more complex blends than do pheromonal blends (Mustaparta 1984).

Preference of *D. confusus* for damaged or felled trees may indicate attraction to ethanol or to other degradation metabolites (Graham 1968; Moeck 1970), which act synergistically with monoterpenes in several solytid species (Renwick and Vité 1969; Borden et al. 1980b; Fatzinger 1985, Tilles et al. 1986; Hunt and Raffa 1989; Chénier and Philogène 1989).

Because (-)-α-pinene is cheaper and more stable than myrtenal, it could have practical potential for operational use. It could be used to improve the sensitivity of semiochemical-baited traps for monitoring or to improve the efficacy of mass trapping. An untested possibility would be to attract *D. confusus* to trees other than *A. lasiocarpa*. However, the effectiveness of the 9:1 blend of (+)EXOB:(+)ENDOB alone as a tree bait (Section 2.3), and the recent discovery that host kairomones are unnecessary in tree baits for the mountain pine beetle (Borden et al. 1993), suggests that it would have little potential for use in containment and concentration (Stock et al. 1993) of *D. confusus* infestations.

4. AGGREGATION PHEROMONES IN *DRYOCOETES AFFABER*: STEREOISOMERISM AND SPECIES SPECIFICITY.

4.1 INTRODUCTION

Dryocoetes affaber (Mann.) is the most widespread member of the genus in North America, ranging from Alaska and Eastern Canada to New Mexico and North Carolina (Bright 1963, 1976). It infests *Picea* spp., and has been reported from *Abies*, *Pseudotsuga*, *Larix* and *Pinus* spp. The males are polygynous and are the first to attack the bole of weakened trees (Keen 1952; Furniss and Carolin 1977). The life cycle is poorly known but in Colorado it appears to have one generation per year and overwinters as adults (McCambridge and Knight 1972). *D. affaber* is often present in large numbers in hosts infested by the spruce beetle, *Dendroctonus rufipennis* (Kirby), and is involved in mortality due to interspecific competition (McCambrige and Knight 1972).

D. confusus is sympatric with *D. affaber* in the subalpine forests of British Columbia, wherein mixtures of (+)EXOB and (+)ENDOB at a 9:1 ratio optimally attract *D. confusus* (Section 2.2). At a 1:1 ratio, either with (±) pheromones or (+) enantiomers, *D. affaber* was attracted in

place of *D. confusus*, which suggested the existence of a reproductive isolation mechanism based on the chirality and the ratio of EXOB and ENDOB.

The objectives of this section were:

1) to isolate and identify the major volatiles produced by *D. affaber*,
2) to determine the most attractive combination of these insect-produced volatiles,
3) to investigate the role of chirality in the system,
4) to elucidate mechanisms of pheromone-based species specificity between *D. confusus* and *D. affaber*.

4.2 MATERIALS AND METHODS

Collection of Insects and Hosts. Bolts of Engelmann spruce, *Picea engelmannii* Parry, both healthy and infested with *D. affaber*, were obtained from trees felled near Merritt, British Columbia. The infested bolts were placed in screened cages in the laboratory at 20-21^{o}C. Emerging beetles were sexed and kept on moistened paper at 5^{o}C until used in laboratory bioassays.

Collection and Analysis of Volatiles. Groups of male or female beetles were placed individually in preformed entrance holes in fresh spruce logs. The beetles were allowed to bore into the phloem tissue for 3 days and were then excised from the phloem. Extracts were prepared by crushing whole beetles in pentane held over dry ice; the liquid fraction was recovered and stored at -27°C. Another batch of spruce logs was infested in the laboratory with male or female *D. affaber*, and placed in aeration chambers. The emanating volatiles were captured on Porapak-Q and recovered by pentane extraction (Pierce et al. 1981).

The whole-body extracts and the volatiles from infested logs were analyzed by GC using Hewlett Packard 5830A and 5880A instruments equipped with capillary inlet systems, flame ionization detectors and open tubular glass columns (30 m x 0.5 mm I.D.) coated with SP-1000 (Supelco, Bellefonte, Pennsylvania). The temperature program was 70°C for 2 min, then 4°C/min. to 180°C holding for 20 min. The enantiomeric composition of EXOB and ENDOB was determined by analysis of the volatiles from feeding-male whole body extracts on a Chirasil-Dex (8) column (25 m x 0.25 mm I.D.) (V. Schurig, University of Tübingen, Germany). Coupled gas chromatography-mass spectrometry (GC-MS) was performed with a Hewlett Packard 5895A GC-MS fitted with a fused silica column (30 m x 0.33 mm I.D.) coated with SP-1000 (J & W

Scientific, Inc., Folsom, California). Helium was the carrier gas for the GC and GC-MS.

Synthetic Pheromones. (±)EXOB (96.3 % pure with 2.5 % ENDOB) and (±)ENDOB (96.4 % pure with 0.4 % EXOB), were obtained from Phero Tech Inc., Delta, B.C. Optically pure brevicomins were synthesized by B.D. Johnston (Dept. of Chemistry, Simon Fraser University), according to the procedures developed by Johnston and Oehlschlager (1982) and Oehlschlager and Johnston (1987); formulations included (+)ENDOB (98.8 % and 90.15 % chemically and optically pure, respectively), (-)ENDOB (97.8 % and 91 % chemically and optically pure, respectively), (-)EXOB (96.4 % and 92.6 % chemically and optically pure respectively). For field experiments conducted in 1992, we also employed (+)EXOB (98.79 and 94.0 % chemically and optically pure, respectively). The Sharpless asymmetric dihydroxylation (Sharpless et al. 1991) was used for the synthesis (E.K. Czyzewska, Dept. of Chemistry, Simon Fraser University, unpublished). Blends of EXOB and ENDOB were prepared by weight, and ratios referred to below are on a weight to weight basis.

Determination of Ratio in Vapor Phase. The ratio in vapor phase was determined for the formulation 1:2 of (±)EXOB:(±)ENDOB. Two glass capillary tubes (1.0 mm I.D.) sealed at one end, containing 12 μL of the 1:2 blend, were kept inside open 400 μL polyethylene tubes at 24 to 26°C. Vapor phase samples were taken from the plastic tube at 24, 40, 48 and 70 h after formulation, and analyzed by GC as above.

Laboratory Bioassays. Experiments on responses to EXOB and ENDOB and their blends were performed using walking beetles in an open arena olfactometer (Wood and Bushing, 1963; Stock and Borden, 1983). Groups of 10 beetles (total 50) of either sex were exposed for 2.5 min to an air stream (500 mL/min.) containing volatile stimuli applied in 10 μL of pentane to a filter paper wick. The solvent was used as a control. Room temperature was 20-21°C and room lighting was diffuse and of low intensity (22.57 lux).

A series of 1 pg stimuli consisting of (+)EXOB, (+)ENDOB, and binary blends of these compounds at ratios of 3:1, (the natural ratio), 2:1, 1:1 (found attractive in field experiments Figs. 3, 5), 1:2, 1:3 and 1:6 were tested. I tried to cover a wide range of possible combinations excluding those with high content of (+)EXOB, considered atractive for *D. confusus* (Section 2.2).

Field Experiments. Trapping experiments were conducted in a forest of Engelmann spruce and subalpine firs *Abies lasiocarpa* (Hook.) Nutt., 40 km West of Merritt, B.C. Multiple funnel traps (Lindgren, 1983; Phero Tech Inc., Delta, B.C.), were placed 15 m apart in randomized complete blocks, with 9 to 20 replicates per experiment. The release rates of synthetic pheromones, either as single compounds or as blends, were approximately 0.2 mg/24 h at 20°C from each glass capillary (1.0 mm I.D.), as determined in the laboratory (Stock 1991). In the forest, the release rates are temperature dependent.

Field Experiment (Exp.) 4A, conducted in 1992, tested blends of (+)EXOB and (+)ENDOB at the following ratios: 3:1 (found in the aerations of male infested logs); 1:1 (attractive for *D. affaber*, Section 2.2); 1:2 (from the results of laboratory bioassays); 1:10 (attractive to *D. affaber* in other field experiments (J.H. Borden[1], pers. comm.); and an unbaited control.

Field Exp. 4B-4D, investigated the question of enantioselectivity. In 1991 Field Exp. 4B tested the

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[1]Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.

attractiveness of (±), (-) and (+) ENDOB, and blends of EXOB:ENDOB in the following combinations of enantiomers (±):(±), (±):(-) and (±):(+).

Field Exp. 4C and 4D, in 1992, tested EXOB:ENDOB blends of (-):(+), (+):(+) and (±):(+) (Field Exp. 4C), and (-):(-), (+):(-) and (±):(+) (Field Exp. 4D). The (±):(+) combination was always present in Field Exps. 4B-4D. In all cases blends of EXOB:ENDOB were in a 1:1 ratio.

Field Exp. 4E utilized the best blends of EXOB:ENDOB established in previous experiments for *D. affaber* and *D. confusus* and challenged their capacity to maintain species specificity. I used (+)EXOB: (+)ENDOB at a 9:1 ratio for *D. confusus*, and (±)EXOB:(+)ENDOB at a 1:1 ratio (i.e. a 1:2 ratio of (+) enantiomers) for *D. affaber*.

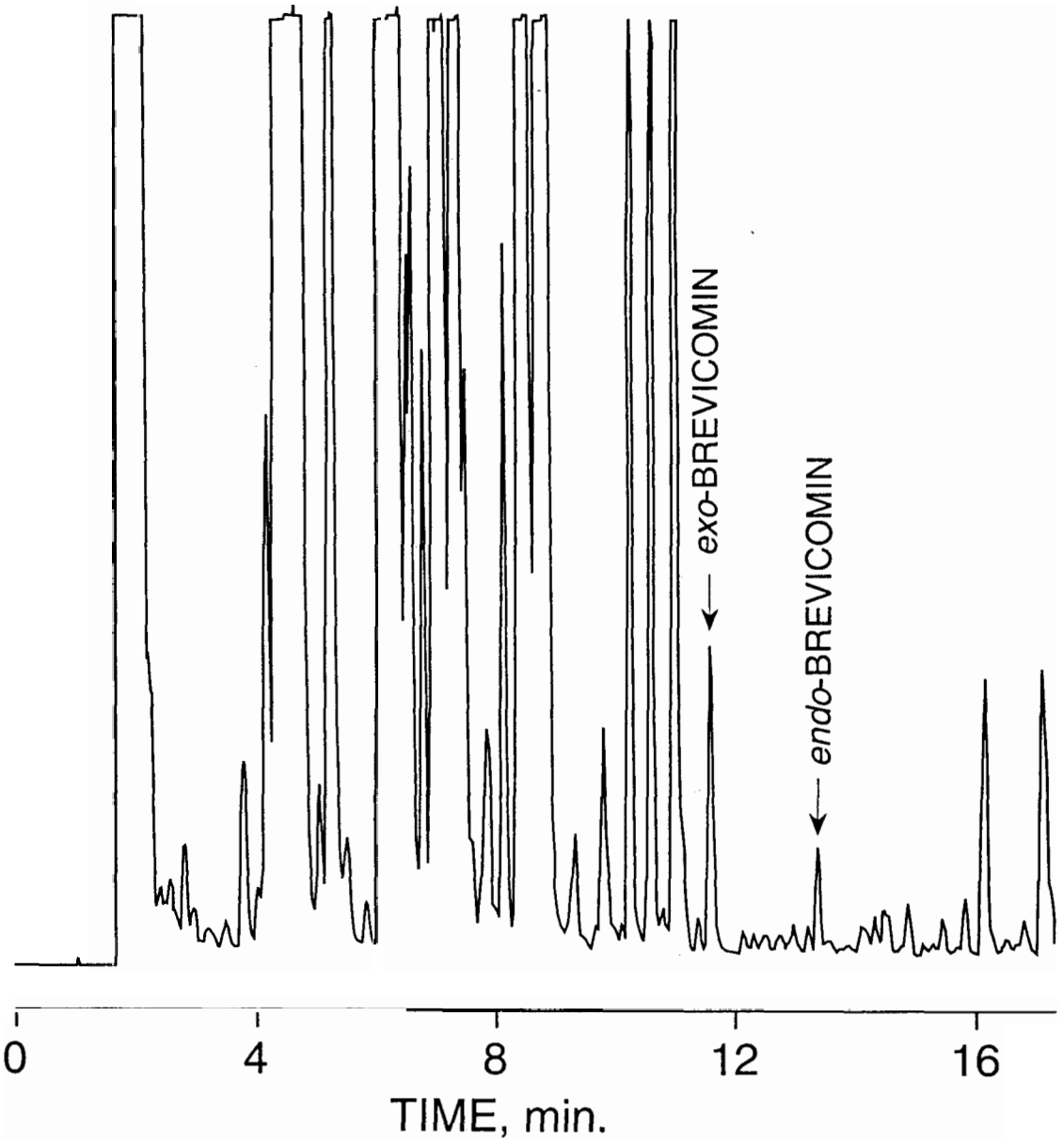
Seasonal Flight Pattern. Field Exp. 2B (Section 2.2.2) was set up in 1991 as soon as the roads were open on 4 June; traps were left in the forest throughout the summer until the first snowfall on 25 September. They were rebaited every 15 days during warm periods and every 20 to 30 days during cooler periods. Five collections of beetles were made with 9 to 45-day intervals between collections.

Statistical Analysis. Laboratory bioassay results were analyzed by one-way analysis of variance (ANOVA) and the Ryan-Einot-Gabriel-Welsch multiple F or "REGWF" test (Schlotzhauer and Littell 1987) utilizing percentages of positive responders converted to $p'=\arcsin\sqrt{p}$, to approximate a normal distribution (Zar 1984). Percent values of 0% were recorded as 1/4 n to improve the transformation (Bartlett 1937). For field trapping experiments I used two-way ANOVA and the REGWF test on numbers of beetles captured transformed by $x'=\log(x+1)$, to remove heteroscedasticity (Zar 1984). In all cases $\alpha=0.05$. Treatments with zero catches were excluded from statistical analyses. All analyses employed SAS computer software (SAS Institute 1990).

4.3 RESULTS AND DISCUSSION

Identification of Candidate Pheromones. (+)EXOB and (+)ENDOB in a 1.7:1 ratio were conspicuous insect-produced compounds found by GC-MS analysis in whole-body extracts of males. Volatiles emanating from male-infested logs also contained (+)EXOB and (+)ENDOB; in this case the ratio was 3.04:1 (Fig. 17). Small amounts of EXOB of undetermined chirality were detected from feeding females in logs.

Figure 17. Gas-liquid chromatogram of Porapak Q-trapped volatiles produced by male *Dryocoetes affaber* feeding in fresh bolts of *Picea engelmannii*, showing *exo*-brevicomin and *endo*-brevicomin in a 3:1 ratio.


exo-BREVICOMIN
endo-BREVICOMIN
0
4
8
12
16
TIME, min.

In laboratory bioassays, blends of (+)EXOB: (+)ENDOB in the ratio range between 2:1 and 1:2, elicited the highest levels of response from female *D. affaber*; males showed some preference for blends in the ratio range between 3:1 and 1:2 (Fig. 18). Both sexes showed the highest numerical response to the 1:2 ratio. The response to (+)EXOB or to (+)ENDOB presented individually was very low.

Ratio in Vapor Phase. The 1:2 blend of (±)EXOB:(±)ENDOB formulated by weight was confirmed by GLC analysis. The ratios ($\bar{X}$±SD) determined by GLC analyses of vapor phase samples after 24, 40, 48 and 70 h were: 1:1.3±0.02, 1:1.7±0.03, 1:1.6±0.02, and 1:1.7±0.02 respectively. Differential volatility of EXOB and ENDOB caused only minor changes from the formulated ratio in the proximity of the release device. Modifications of this ratio are to be expected at further distances from the release point as a result of diverse environmental factors (e.g. temperature, turbulence) that affect the pheromone plume in the forest (Murlis et al. 1992).

Field Experiments. The highest response from both males and females in Field Exp. 4A was elicited by the (+):(+) blend at the 1:2 ratio (Fig. 19). As in the laboratory (Fig. 18), the natural 3:1 blend was poorly attractive.

Figure 18. Response of *Dryocoetes affaber* in laboratory bioassays to 1 pg stimuli of (+)-*exo*-brevicomin (EXOB), (+)-*endo*-brevicomin (ENDOB) and six blends of the two isomers at different ratios. Response to pentane controls: males 4 %, females 6 %. Percents with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh multiple F test, $P<0.05$.

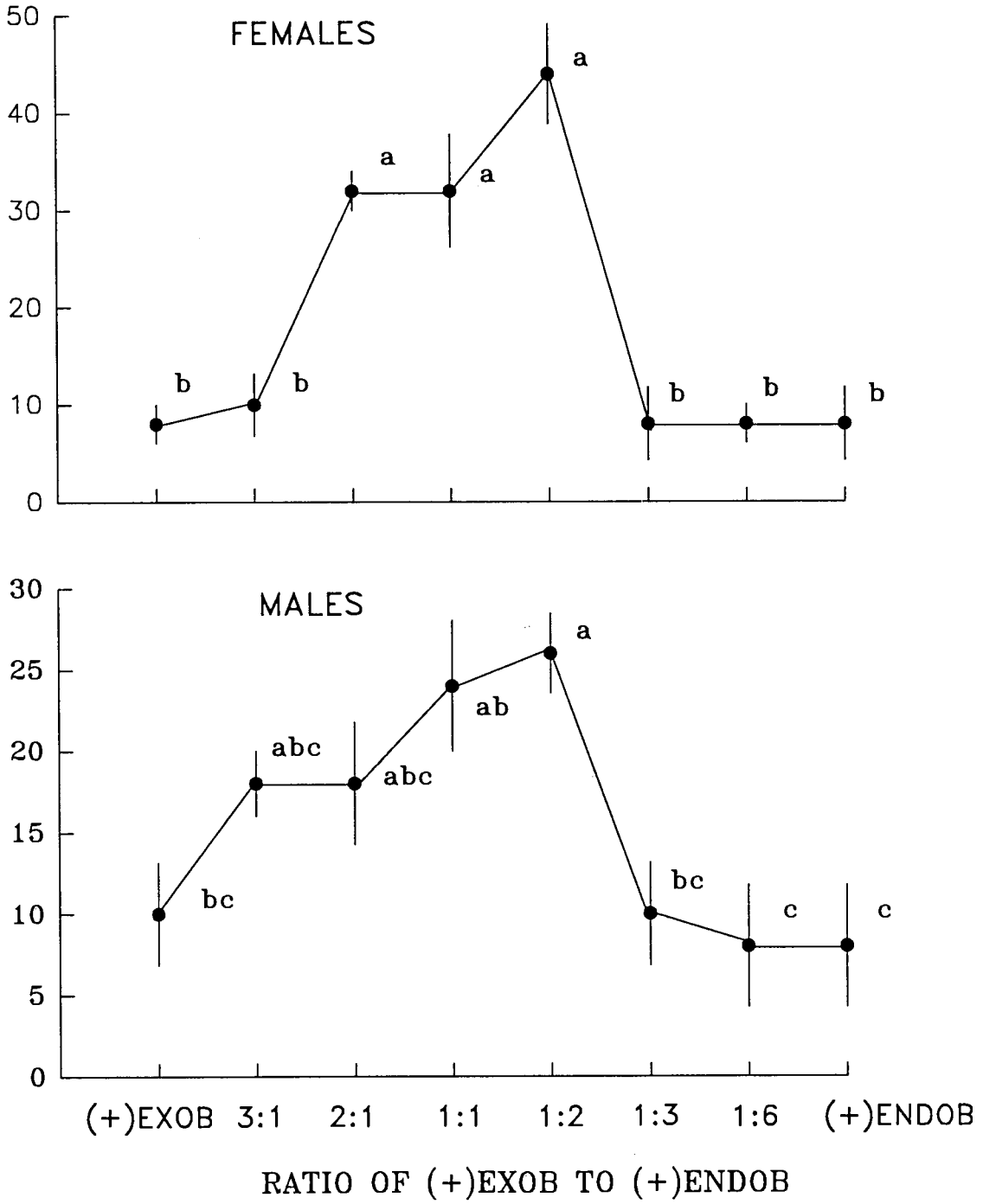
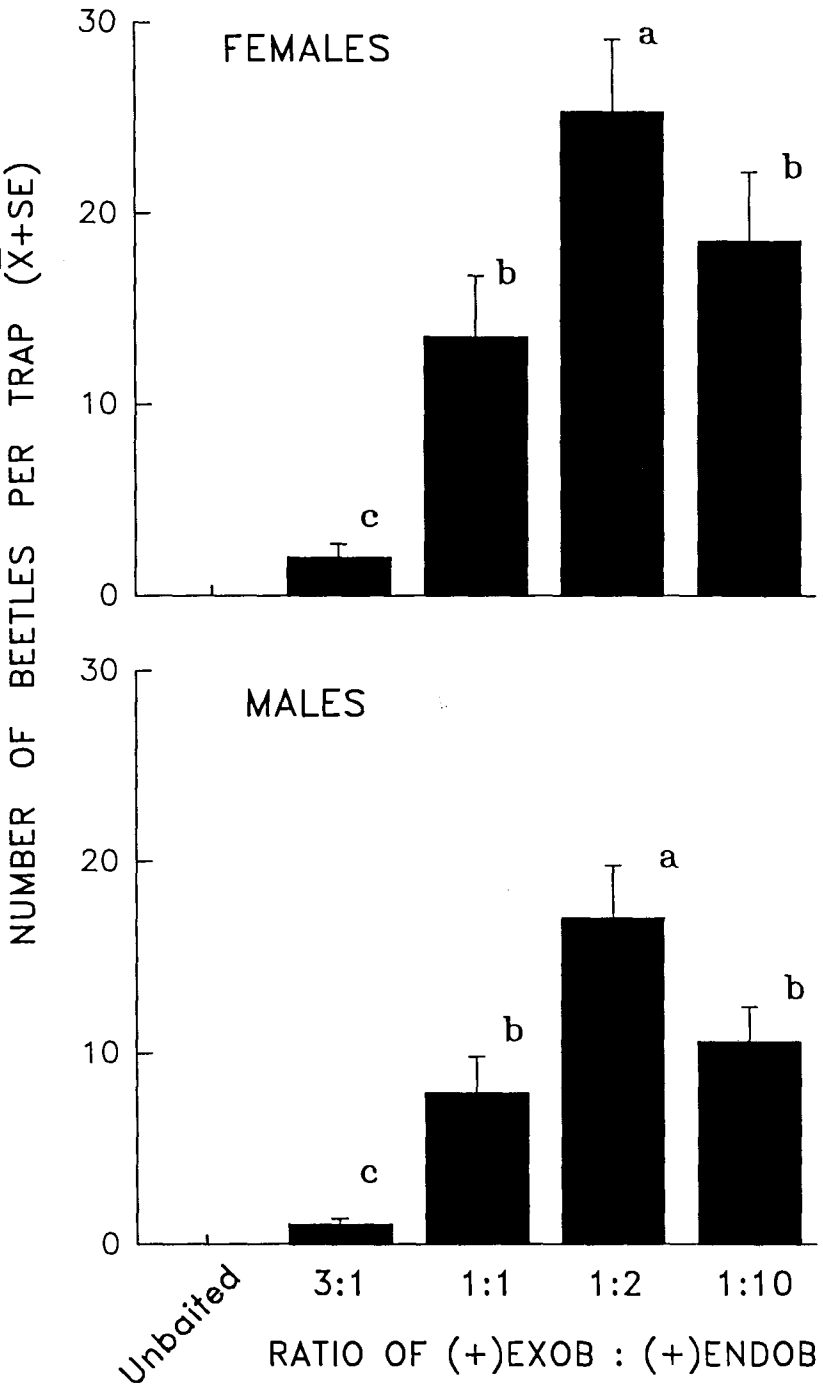

FEMALES
50
40
30
20
10
0
b
b
a
a
a
b
b
b
MALES
30
25
20
15
10
5
0
bc
abc
abc
ab
a
bc
c
c
(+)EXOB
3:1
2:1
1:1
1:2
1:3
1:6
(+)ENDOB
RATIO OF (+)EXOB TO (+)ENDOB

Figure 19. Numbers of *Dryocoetes affaber* caught in Field Exp. 4A to traps baited with blends of (+)-*exo*-brevicomin (EXOB) and (+)-*endo*-brevicomin (ENDOB) in four ratios; 10 replicates, 7 July to 5 August, and 10 replicates, 5 to 20 August, 1992. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh multiple F test, $P<0.05$.


FEMALES
MALES
NUMBER OF BEETLES PER TRAP (X̄+SE)
30
20
10
0
a
b
b
c
a
b
b
c
Unbaited
3:1
1:1
1:2
1:10
RATIO OF (+)EXOB : (+)ENDOB

Blends of 1:1 and 1:10 (+)EXOB:(+)ENDOB attracted significantly fewer *D. affaber* of both sexes than did the 1:2 blend.

For lepidoptera, it is generally accepted that optimum blends of pheromone components closely approximate the natural ratio emitted by the producing sex. However, production and reception genes are not linked (Roelofs et al. 1987). Response to different blends could indicate missing elements in the chemical message (Baker 1989). It is possible that environmental factors could alter the pheromone plume, from the natural 3:1 ratio to more attractive ratios. Other effects such as geographical and individual variation (Miller et al. 1989), physiological changes due to manipulation and storage, or mechanisms of avoidance of competition for pheromonal channels, could help to explain the observed difference between pheromone production and response in *D. affaber*.

These results suggest that in *D. affaber* there is considerable tolerance to variation in ratios of pheromone components, as reported for other bark beetles (Schlyter et al. 1987; Byers 1988), and to a lower degree in moths (Linn and Roelofs 1989). Tolerance to variation in pheromone quantity and component ratios may have evolved on the basis of the rather large variation in monoterpene precursors

(Byers 1989). Pheromonal plasticity could be of selective advantage for secondary bark beetles. For *D. affaber*, plasticity would be restricted to EXOB:ENDOB blends that comprised >50 % ENDOB.

There was a decrease in response to ENDOB by both sexes in Field Exp. 4B, from (+) to (±) and finally (-), indicating that the (+) enantiomer is the active component; response to the blends indicate that the antipode is inhibitory (Fig. 20). The 1:1 blend of (±)EXOB:(+)ENDOB was the most attractive, indicating that synergism occurs between EXOB and ENDOB, but not disclosing which enantiomer of EXOB is active. This question was resolved by Field Exp. 4C, in which (+) or (±)EXOB in combination with (+)ENDOB elicited the highest levels of response (Fig. 21). These results indicate that (+)EXOB is active and that (-)EXOB is inactive. The partial activity of the (-):(+) blend (Fig. 22) can be attributed to the 7.4 % (+)EXOB impurity. In Field Exp. 4D, binary blends containing only (-)ENDOB were not attractive (Fig. 22), confirming that (+)ENDOB is the active enantiomer.

To facilitate interpretation of enantioselectivity, the pooled results of Field Exp. 4B-4D (all conducted in the same forest stand) were plotted as proportions

Figure 20. Numbers of *Dryocoetes affaber* caught in Field Exp. 4B to traps baited with (+), (-) and (±)-*endo*-brevicomin (ENDOB), and blends of (±)-*exo*-brevicomin (EXOB) and (ENDOB) mixed in a 1:1 ratio, in three enantiomeric combinations, 9 replicates, 9 August to 25 September, 1991. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh multiple F test, $P<0.05$.

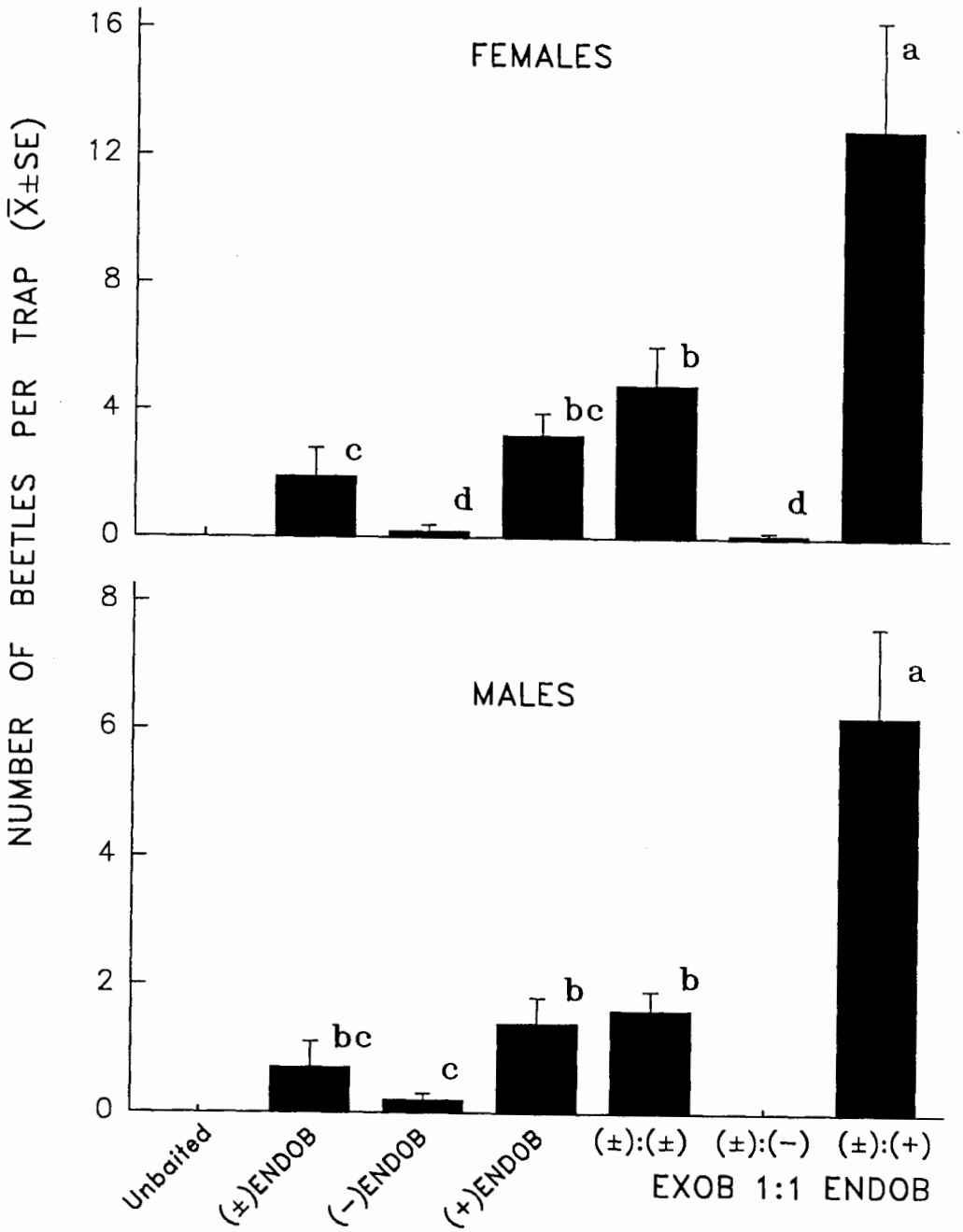

NUMBER OF BEETLES PER TRAP (X̄±SE)
FEMALES
MALES
16
12
8
4
0
8
6
4
2
0
a
b
bc
c
d
d
a
b
b
bc
c
Unbaited
(±)ENDOB
(−)ENDOB
(+)ENDOB
(±):(±)
(±):(−)
(±):(+)
EXOB 1:1 ENDOB

Figure 21. Numbers of *Dryocoetes affaber* caught in Field Exp. 4C to traps baited with chiral combinations of *exo*-brevicomin (EXOB) and (+)-*endo*-brevicomin (ENDOB) all in a 1:1 ratio, 10 replicates, 16 June to 7 July, and 10 replicates, 7 to 23 July, 1992. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh multiple F test, $P<0.05$.

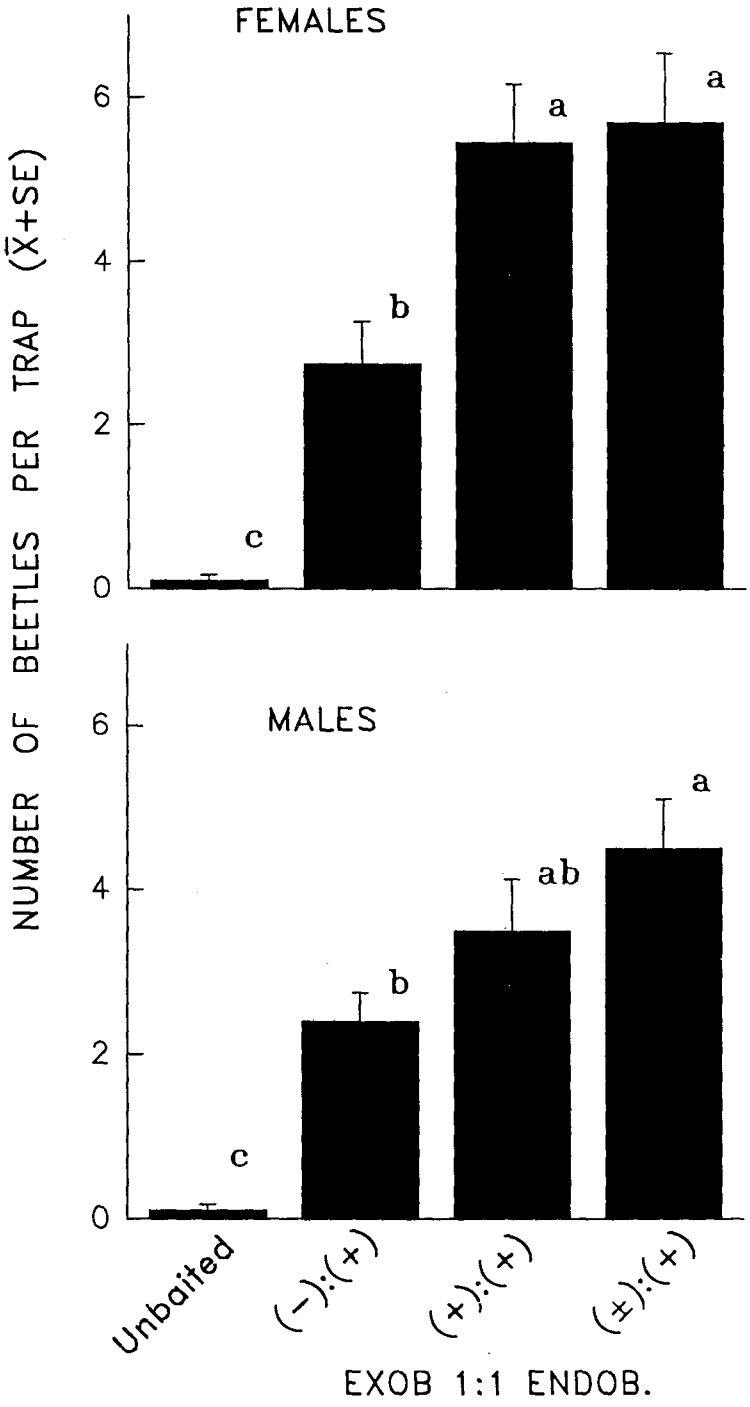
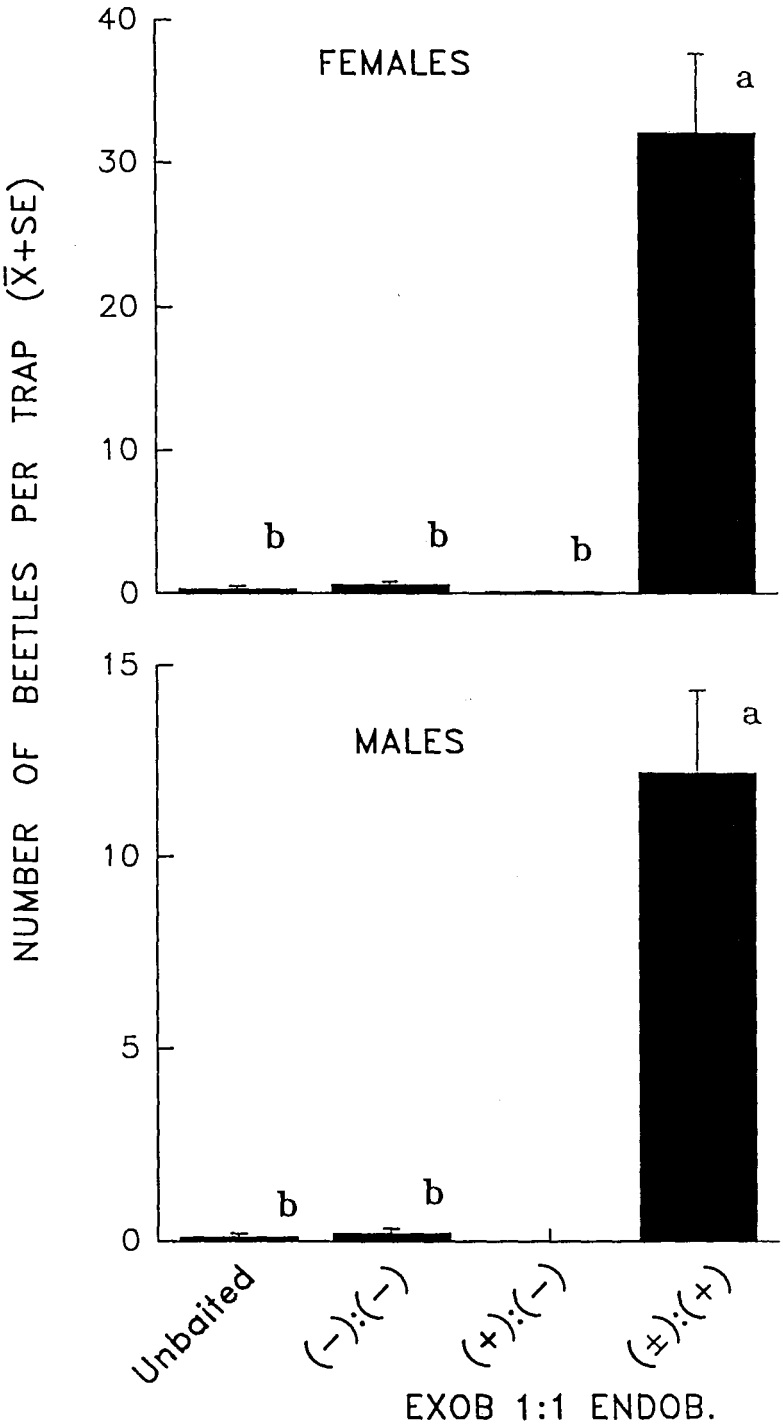

FEMALES
MALES
NUMBER OF BEETLES PER TRAP (X̄+SE)
0
2
4
6
a
b
c
ab
Unbaited
(−):(+)
(+):(+)
(±):(+)
EXOB 1:1 ENDOB.

Figure 22. Numbers of *Dryocoetes affaber* caught in Field Exp. 4D to traps baited with enantiomeric combinations of *exo*-brevicomin (EXOB) and *endo*-brevicomin (ENDOB) all in a 1:1 ratio, 10 replicates, 22 July to 20 August, 1992. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsh multiple F test, $P<0.05$.

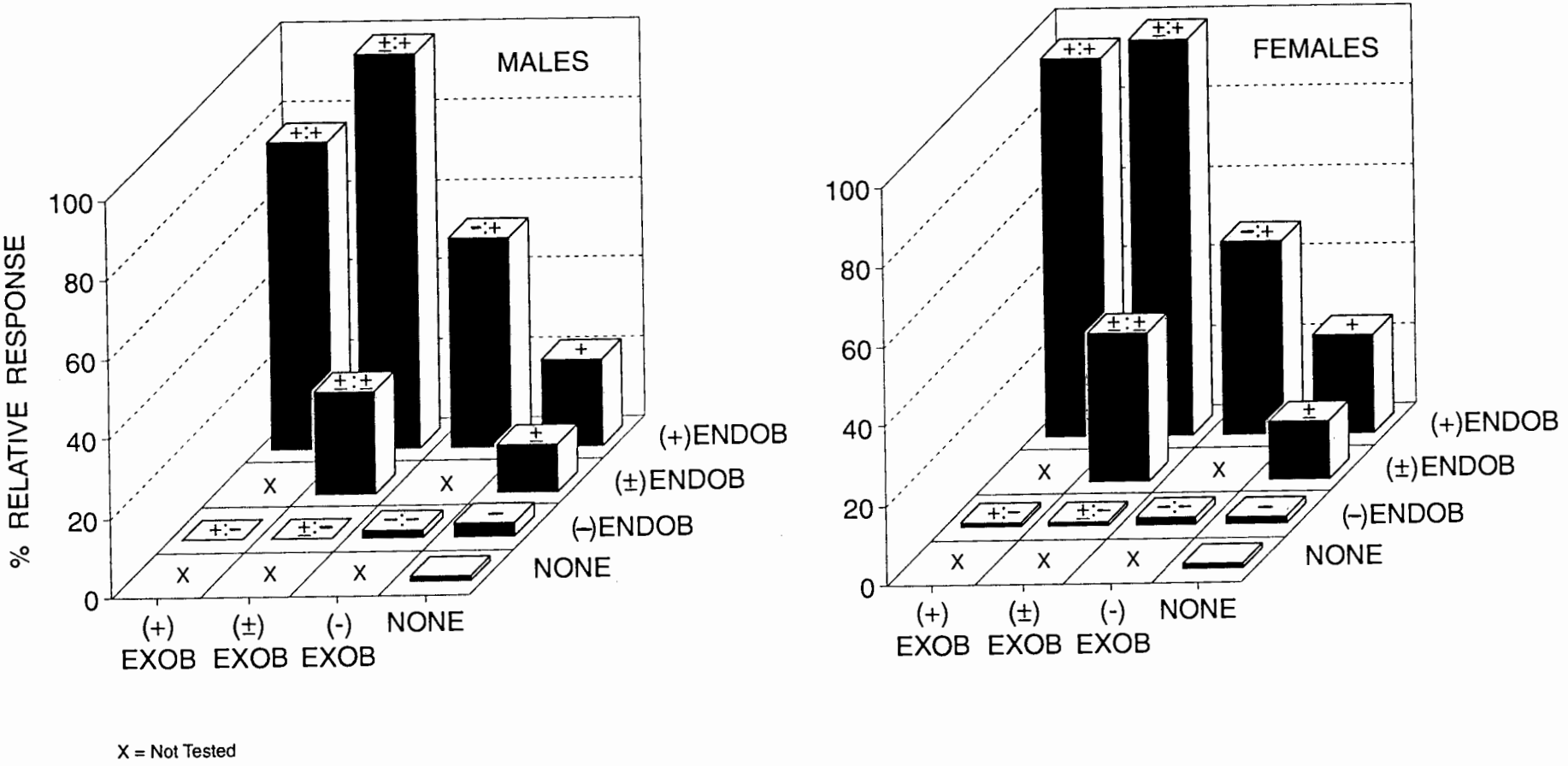

FEMALES
MALES
NUMBER OF BEETLES PER TRAP (X̄+SE)
40
30
20
10
0
15
10
5
0
a
b
b
b
a
b
b
Unbaited
(−):(−)
(+):(−)
(±):(+)
EXOB 1:1 ENDOB.

(percentages), with response to the most attractive treatment [(±)EXOB:(+)ENDOB in a 1:1 ratio] normalized to 100 % (Fig. 23). It should be noted that a 1:1 ratio of (±)EXOB:(+)ENDOB results in a 1:2 ratio of the active (+) enantiomers, the most attractive ratio of geometrical isomers found in Field Exp. 4A (Fig. 19). Fig. 23 shows evidence for the combined effect of optical and geometrical isomerism. (+)ENDOB (top row) is revealed to be the major component in the chemical signal. The capacity for (-)ENDOB to cause an inhibition of response to its antipode is shown in the second row from the top (Fig. 23).

Synergism between (+)EXOB and (+)ENDOB, either as (+):(+) or (±):(+) blends, is disclosed in the two columns on the left of Fig. 23. (+)EXOB is multifunctional, as at high ratios it is inhibitory (Fig. 18), just as (+)ENDOB is for *D. confusus* (Section 2.2). Co-occurrence of the (-) enantiomers of EXOB and ENDOB has an inhibitory effect for *D. confusus* (Section 2.2) and the European *D. autographus* (Kohnle and Vité 1984). It is probable that a similar effect occurs in *D. affaber* (Fig. 23).

When the best blends of EXOB:ENDOB for *D. confusus* and *D. affaber* (Figs. 18-23) were tested in the same location in Field Exp. 4E, there was a clear demonstration that the responses of the two sympatric *Dryocoetes* spp. to blends of

Figure 23. Summary of pooled results obtained in Field Exp. 4B-4D. Data normalized so that 100 % response occurs to the blend of (±)-*exo*-brevicomin:(+)-*endo*-brevicomin in a 1:1 ratio.


MALES
FEMALES
% RELATIVE RESPONSE
100
80
60
40
20
0
+:+
±:+
−:+
±:±
+
±
+:−
±:−
−:−
−
X
(+)ENDOB
(±)ENDOB
(−)ENDOB
NONE
(+) EXOB
(±) EXOB
(−) EXOB
NONE

X = Not Tested

EXOB:ENDOB were highly species-specific (Fig. 24). The numbers of *D. confusus* captured in response to the 1:2 blend, and of *D. affaber* attracted by the 9:1 blend were not statistically different from the captures obtained with unbaited traps (Ryan-Einot-Gabriel-Welsh multiple F test, $P<0.05$). These results support the hypothesis of semiochemical-based reproductive isolation, and I conclude that a mechanism of pheromonal exclusion based on the ratio of EXOB:ENDOB and on discrimination of enantiomers exists between *D. affaber* and *D. confusus*.

Seasonal Flight Pattern. The results from the binary blend at the 1:1 ratio in Field Exp. 2B, provided a profile of the flight pattern of *D. affaber* (Fig 25). Low temperatures in the high altitude forest (1540 m), make it unlikely that insects would fly before snow melting is complete at the end of May. Captures of *D. affaber* rose to a peak in flight activity by mid July to early August; captures continued until well into September. The proportion of males in the catches declined from 55 % up to 4 July to <50 % after 17 July. The results from other trapping experiments are consistent with this flight pattern.

According to Stock (1991), the sympatric *D. confusus* has a bimodal flight pattern with peaks in flight activity in mid- to late-June, and then in mid-August (data from two

Figure 24. Numbers of *Dryocoetes affaber* and *D. confusus* caught in Field Exp. 4D to traps baited with optimal blends for each species of *exo*-brevicomin (EXOB) and *endo*-brevicomin (ENDOB), 10 replicates, 16 June to 7 July, 1992. Bars with the same letter within each species are not significantly different, Ryan-Einot-Gabriel-Welsh multiple F test, $P<0.05$. Note that a 1:1 ratio of (±)EXOB:(+)ENDOB results in a 1:2 ratio of (+) enantiomers.

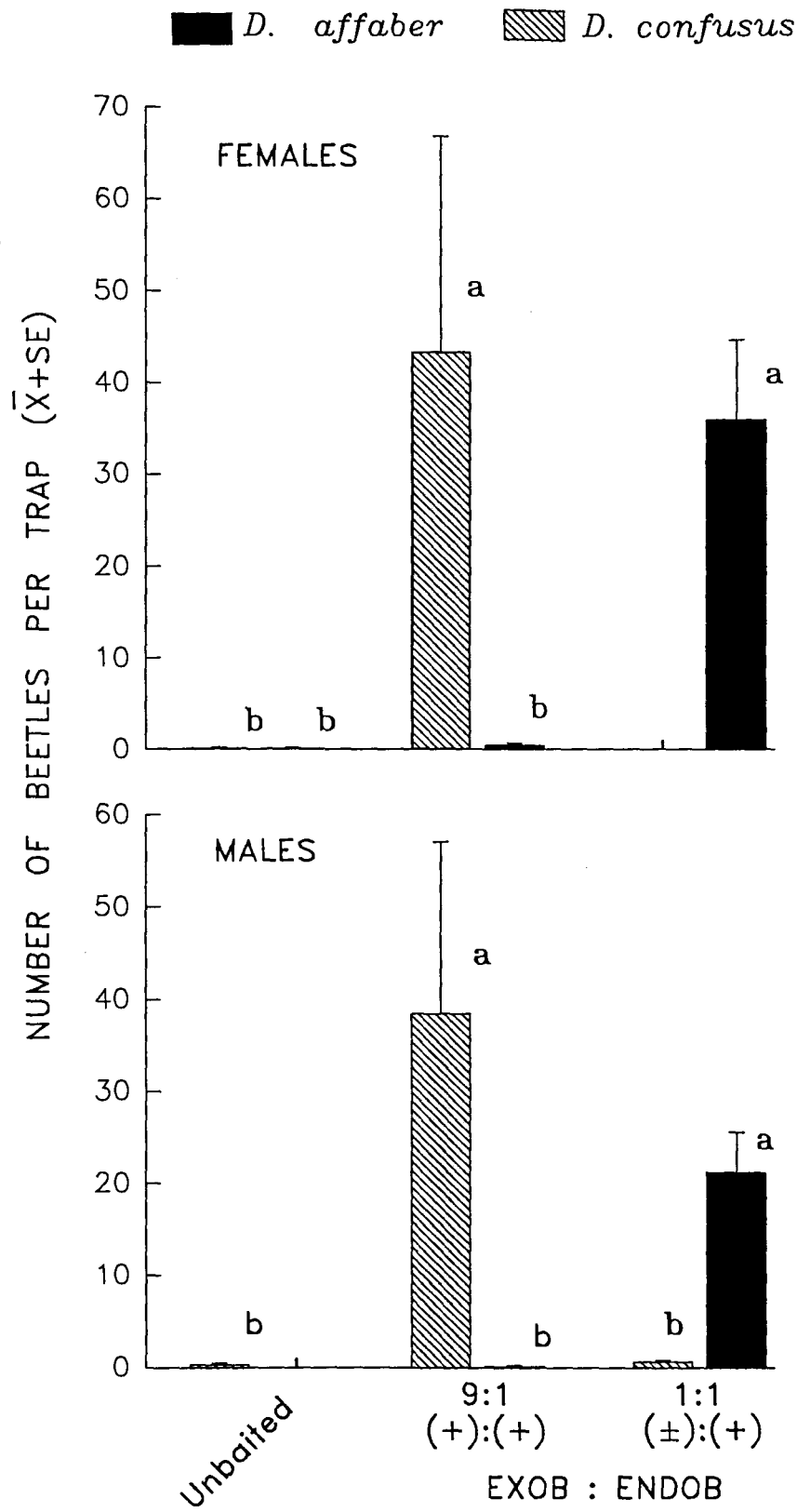
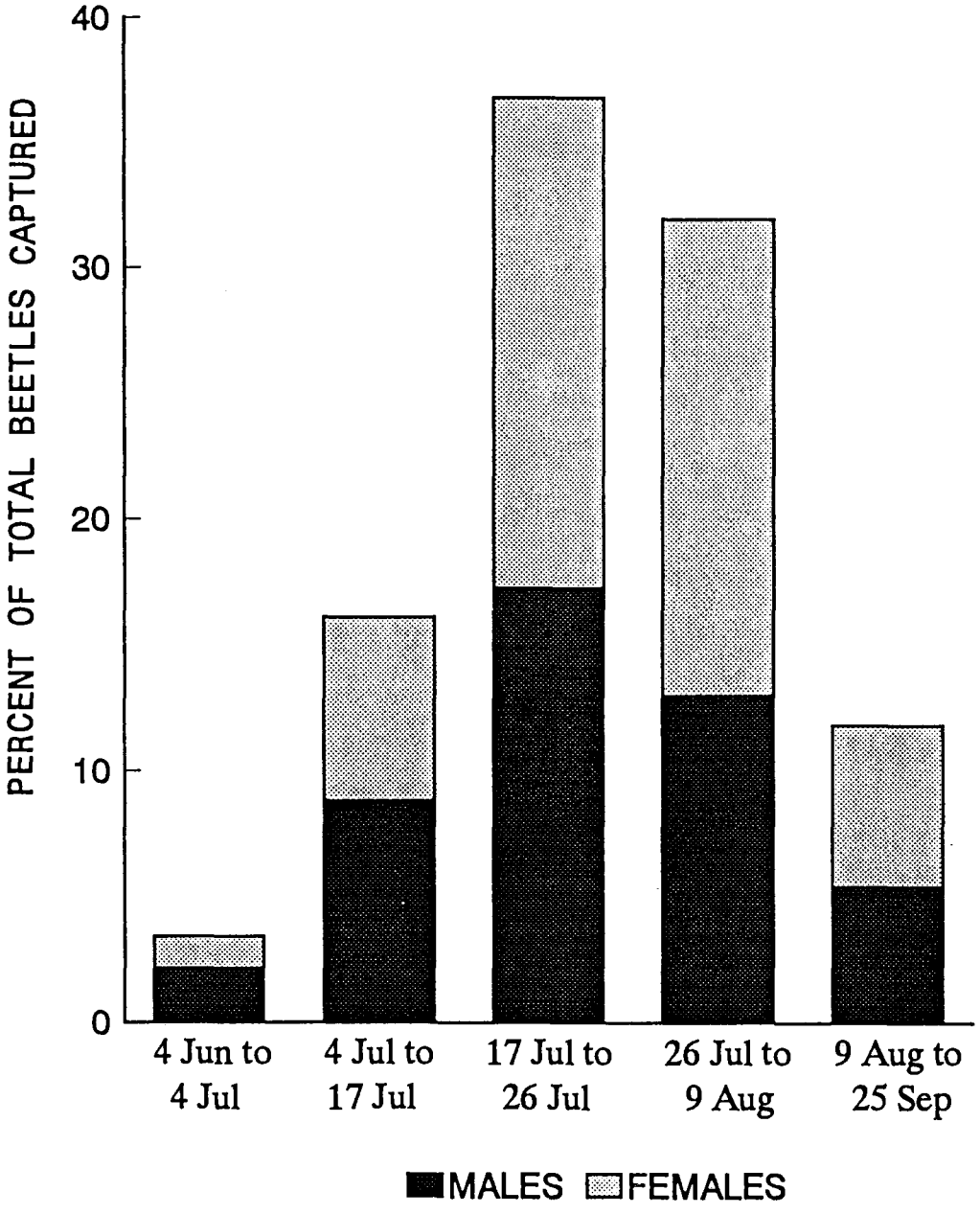

D. affaber
D. confusus
FEMALES
MALES
NUMBER OF BEETLES PER TRAP (X̄+SE)
70
60
50
40
30
20
10
0
a
b
Unbaited
9:1
(+):(+)
1:1
(±):(+)
EXOB : ENDOB

Figure 25. Seasonal flight pattern of *Dryocoetes affaber* during the summer of 1991, in Prospect Ck., Merritt, B.C.. Proportions of beetles caught in Field Exp. 2B to traps baited with (+)EXOB:(+)ENDOB at the 1:1 ratio. Total number of beetles caught, 1200.


PERCENT OF TOTAL BEETLES CAPTURED
40
30
20
10
0
4 Jun to 4 Jul
4 Jul to 17 Jul
17 Jul to 26 Jul
26 Jul to 9 Aug
9 Aug to 25 Sep
MALES
FEMALES

locations in North-central B.C). The sequence of collections from Field Exp. 2B, did not allow for proper determination of the *D. confusus* flight pattern, especially from 17 July to 9 August. However, if a bimodal pattern occurs for *D. confusus* in southern B.C., there would be some degree of temporal separation between the two congenerics, reinforcing pheromonal exclusion as a mechanism of resource partitioning and reproductive isolation.

D. affaber is not sympatric with *D. confusus* over much of its range (Bright 1963, 1976). The evolutionary forces operating in the development of fine tuning of pheromone channels when closely related species are in sympatry would not exist in allopatry. Therefore, I hypothesize that character displacement of pheromones could occur in *D. affaber* where it is sympatric with *D. confusus*. Studies on *D. affaber* pheromones in other areas might well disclose considerable variation in the production of and response to pheromones.

5. SECONDARY ATTRACTION IN *PITYOKTEINES MINUTUS* AND SEMIOCHEMICAL INTERACTIONS WITH TWO *DRYOCOETES* SPECIES.

5.1 INTRODUCTION

The engraver beetle *Pityokteines minutus* colonizes dying or declining *Abies lasiocarpa* and Douglas fir, *Pseudotsuga menziesii*, in southern British Columbia and western Alberta, south to Arizona and New Mexico (Bright 1976; S.L. Wood 1982). As are other American congenerics, *P. minutus* is not considered economically important. There is only fragmentary information available on its biology.

In Ontario, *P. sparsus* (LeConte) was abundant in dead or dying balsam fir, *Abies balsamea* (L.) Mill., affected by spruce budworm defoliation. Attack on still living trees was lighter than in dead trees, and was usually confined to the lower parts of the trunk. Beetles entering the hosts by mid-May had completed development and emerged in September; brood adults attacked other trees, cut feeding galleries and overwintered therein (Belyea 1951). In Europe, *Pityokteines* spp. are a chronic problem in fir forests and can attain outbreak levels (Viebig 1948; Kraemer 1950; Sekendiz 1987).

Evidence of secondary attraction in the genus was first observed in the European *P. curvidens* Germ.; male frass attracted females in the laboratory (Hierholzer 1950). Logs infested with male *P. spinidens* Reitt, attracted conspecific females (Chararas 1968). Ipsenol (2-methyl-6-methylene-7-octen-4-ol) was identified from feeding male *P. curvidens* (Harring et al. 1975). In field tests *P. curvidens* were attracted to sleeve olfactometers baited with (±)- and (*S*)-(-)-ipsenol and not to the antipode; both enantiomers of ipsdienol were inhibitory (Harring and Mori 1977). Males of the sympatric *P. vorontzovi* Jacobs produced ipsenol and ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol), and responded to sleeve olfactometers baited with (±)-ipsenol and (*R*)-(-)-ipsdienol. Males of *P. spinidens* Reitt. produced ipsenol and ipsdienol, but no results were obtained in field tests (Harring 1978).

The study of semiochemical-based communication in *P. minutus* should contribute to a better understanding of the interactive network of bark beetle species in spruce-balsam forests, and could lead to an improved capacity for using semiochemicals in the management of pest species, e.g. by semiochemical-induced competitive displacement of *D. confusus* (Borden 1992). Accordingly, the objectives of this study were:

1) to test the hypothesis that secondary attraction occurs in *P. minutus*,
2) to isolate and identify attractive volatiles produced by *P. minutus*,
3) to determine the role of these compounds in mediating secondary attraction, and
4) to explore the interspecific activity of these compounds on *D. confusus* and *D. affaber*.

5.2 MATERIALS AND METHODS.

Collection of Insects and Hosts. Logs of subalpine fir, both uninfested and infested with *P. minutus*, were obtained from trees felled near Merritt, B.C. The infested logs were placed in screened cages in the laboratory; emerging adults were sexed based on morphological differences in the frons and elytral declivity (Bright 1976), and kept on moistened paper at 5 ^{o}C until used.

Collection, Fractionation and Analysis of Frass and Volatiles. Male and female beetles were confined by gelatin capsules affixed over preformed entrance holes in fresh subalpine fir bolts. They were allowed to bore into the phloem tissue for seven days, after which, five emergent females were allowed to join each male. The frass produced

by males, females, and males plus females, was collected every 12 h kept separated in sealed vials and held at -12°C. When enough frass had accumulated, it was distilled through a 30 cm glass Dufton column. Individual subalpine fir logs were infested as above with 150 male *P. minutus* per log, and placed in aeration chambers. Volatiles from these logs were captured on Porapak-Q for seven days and recovered by extracting it with pentane (Pierce et al. 1981).

The volatiles emanating from infested logs were analyzed by GC using Hewlett Packard 5830A and 5880A instruments equipped with capillary inlet systems, flame ionization detectors and open tubular glass columns (30 m x 0.5 mm ID) coated with SP-1000 (Supelco, Bellefonte, Pennsylvania). The temperature program was 70°C for 2 min, then 4°C/min to 180°C holding for 20 min. The enantiomeric composition of insect-produced volatiles was determined on a Chirasil-Dex (8) column (25 m x 0.25 mm I.D.) (V. Schürig, University of Tübingen, Germany) at 90°C. Coupled gas chromatography-mass spectrometry (GC-MS) was performed with a Hewlett Packard 5895A GC-MS fitted with a fused silica column (30 m x 0.33 mm I.D.) coated with SP-1000 (J & W Scientific Inc., Folsom, California). Helium was the carrier gas for the GC and GC-MS.

Fractionation of volatiles from infested log aerations by micropreparative GC was conducted with a Varian 1200 instrument fitted with a stainless steel column (3.05 m X 3.18 mm O.D.), packed with 10% SP-1000 on Supelcoport (100-120 mesh) (Supelco Inc.), a 10:1 effluent splitter, FID, and a thermal gradient collector (Brownlee and Silverstein 1968). The temperature program was 70°C for 2 min, then 4°C/min to 180°C holding for 5 min.

Synthetic Pheromones. (*S*)-(-)-ipsenol and (*S*)-(+)-ipsdienol [referred to hereafter as (-)ipsenol and (+)ipsdienol], both 97 % optically pure, were obtained from Phero Tech Inc. (Delta, B.C.). (+)ENDOB (81 % and 91 % chemically and optically pure, respectively) was synthesized by B.D. Johnston (Dept. of Chemistry, Simon Fraser University); (+)EXOB (98.8 % and 94 % chemically and optically pure, respectively) was synthesized by E.K. Czyzewska (Dept. of Chemistry, Simon Fraser University).

Laboratory Bioassays. The responses of *P. minutus* to frass extracts, fractions thereof and to synthetic pheromones, were tested using walking beetles in an arena olfactometer (Wood and Bushing 1963; Stock and Borden 1983). Groups of 10 beetles (total 50) of either sex were exposed for 4 min to an air stream (500 mL/min) containing volatile stimuli applied in 10 μL of pentane to a filter paper wick.

The solvent was used as a control. Room temperature was 20-21°C and room lighting was diffuse and of low intensity (22.57 lux).

I evaluated the responses of beetles of both sexes to extracts equivalent to 0.5 mg of frass produced by male, female, and beetles of both sexes. Fractions of extracts of male-produced frass were tested at a concentration equivalent to 0.6 mg of frass. Synthetic (-)ipsenol and (+)ipsdienol were also tested at 1 ng doses.

Field Experiments. Trapping experiments were conducted during the summer of 1993, in forests of subalpine fir and Engelmann spruce infested with *D. confusus* and *D. affaber*, located approximately 40 km west of Merritt, B.C. (altitude 1450-1600 m). Twelve-unit multiple funnel traps (Lindgren 1983; Phero Tech Inc., Delta, B.C.) were placed 15 m apart in randomized complete blocks, with 8 to 15 replicates. Ipsenol and ipsdienol were released at approximately 0.2 mg/day from bubble caps (Phero Tech Inc.) containing the active material at 8 % in butanediol. EXOB and ENDOB were released as blends from glass capillary tubes (1.0 mm I.D.) sealed at one end; the release rates were approximately 0.2 mg/day as determined in the laboratory at 20°C (Stock 1991).

In Field Experiment (Exp.) 5A, emerging *P. minutus* were sexed and allowed to bore into 30 cm-long subalpine fir bolts for 24 h. The experimental bolts were placed into polyester screen bags to prevent entry by other insects, and hung beside a multiple-funnel trap. Treatments were: 50 males per bolt; 50 females per bolt; 50 males and 150 females per bolt; uninfested bolt, and a blank control trap. The traps were reloaded with insects and logs every 14-30 days.

Field Exp. 5B tested the response to compounds identified in male volatiles. The treatments were: (-) ipsenol; (+)ipsdienol; the two compounds together and an unbaited control trap.

Field Exp. 5C, investigated possible pheromonal interactions between *D. confusus* and *P. minutus*. Treatments were: the 9:1 blend of (+)EXOB:(+)ENDOB attractive to *D. confusus* (Section 2.2); (-)ipsenol and (+)ipsdienol (produced by male *P. minutus*); a combination of the two baits; and an unbaited control.

Field Exp. 5D, tested for interactions between *D. affaber* and *P. minutus*. I tested the 1:2 blend of (+)EXOB: (+)ENDOB attractive to *D. affaber* (Section 4); (-)ipsenol

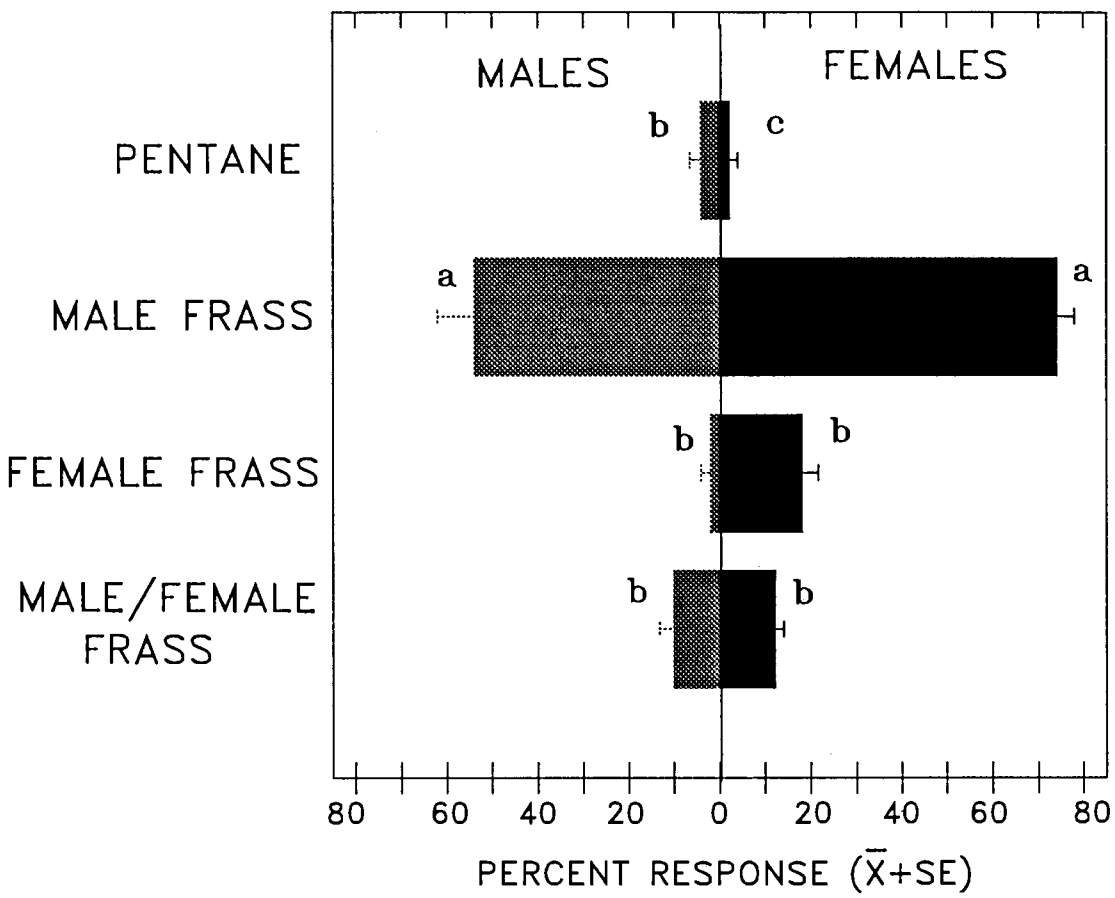
and (+)ipsdienol; a combination of the two baits, and an unbaited control.

Statistical Analysis. Laboratory bioassay results were analized by one-way analysis of variance (ANOVA) and the Ryan-Einot-Gabriel-Welsch multiple F or "REGWF" test (Schlotzhauer and Littell 1987) utilizing percentages of positive responders converted to $p'=\arcsin \sqrt{p}$, to approximate a normal distribution (Zar 1984). Percent values of 0 % were recorded as 1/4 n to improve the transformation (Bartlett 1937). For field trapping experiments I used two-way ANOVA and the REGWF test on numbers of beetles captured transformed by $x'=\log(x+1)$ to remove heteroscedasticity (Zar 1984). If data were not then normally distributed I used the non-parametric Friedman test (Friedman 1937; Conover 1980). In all cases $\alpha=0.05$. SAS computer software (SAS Institute 1990) was employed for the analyses.

5.3 RESULTS AND DISCUSSION

Identification of Candidate Pheromones. *P. minutus* of both sexes responded to male-produced frass (Fig. 26), indicating that feeding males produce an attractive pheromone. Females responded weakly to female-produced

Figure 26. Response of *Pityokyeines minutus* in laboratory olfactometer bioassays to 500 μg stimuli of extracts of frass produced by feeding males, females or both sexes together. Percents with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$


MALES
FEMALES
PENTANE
MALE FRASS
FEMALE FRASS
MALE/FEMALE FRASS
b
c
a
a
b
b
b
b
80
60
40
20
0
20
40
60
80
PERCENT RESPONSE (X̄+SE)

frass, and to frass from galleries containing males and females.

(*S*)-(-)-ipsenol and (*S*)-(+)-ipsdienol in approximately 1.3:1 ratio were conspicuous compounds found by GC-MS analysis of volatiles emanating from male-infested logs. The same two compounds were present (1.1:1 ratio) in male-produced frass (Fig. 27).

Fractionation of male-produced frass yielded three fractions: F1 containing monoterpenes, F2 including (-) ipsenol, (+)ipsdienol and others, and F3 comprised oxygenated terpenes and sesquiterpenes. Olfactometer bioassays disclosed activity in fraction F2, and possibly in F1 (Table 8). Response to combinations including F2 elicited the highest levels of response especially from females, the combination F2 plus F3 and the three fractions recombined were as attractive as the whole extract.

The two major male-specific compounds (-)ipsenol and (+)ipsdienol elicited positive responses from both sexes in the laboratory; females showed an additive response to the combination of the two compounds (Fig. 28).

Figure 27. Gas-liquid chromatogram of volatiles from frass produced by male *Pityokteines minutus* feeding in fresh bolts of *Abies lasiocarpa*, indicating the peaks corresponding to ipsenol and ipsdienol.

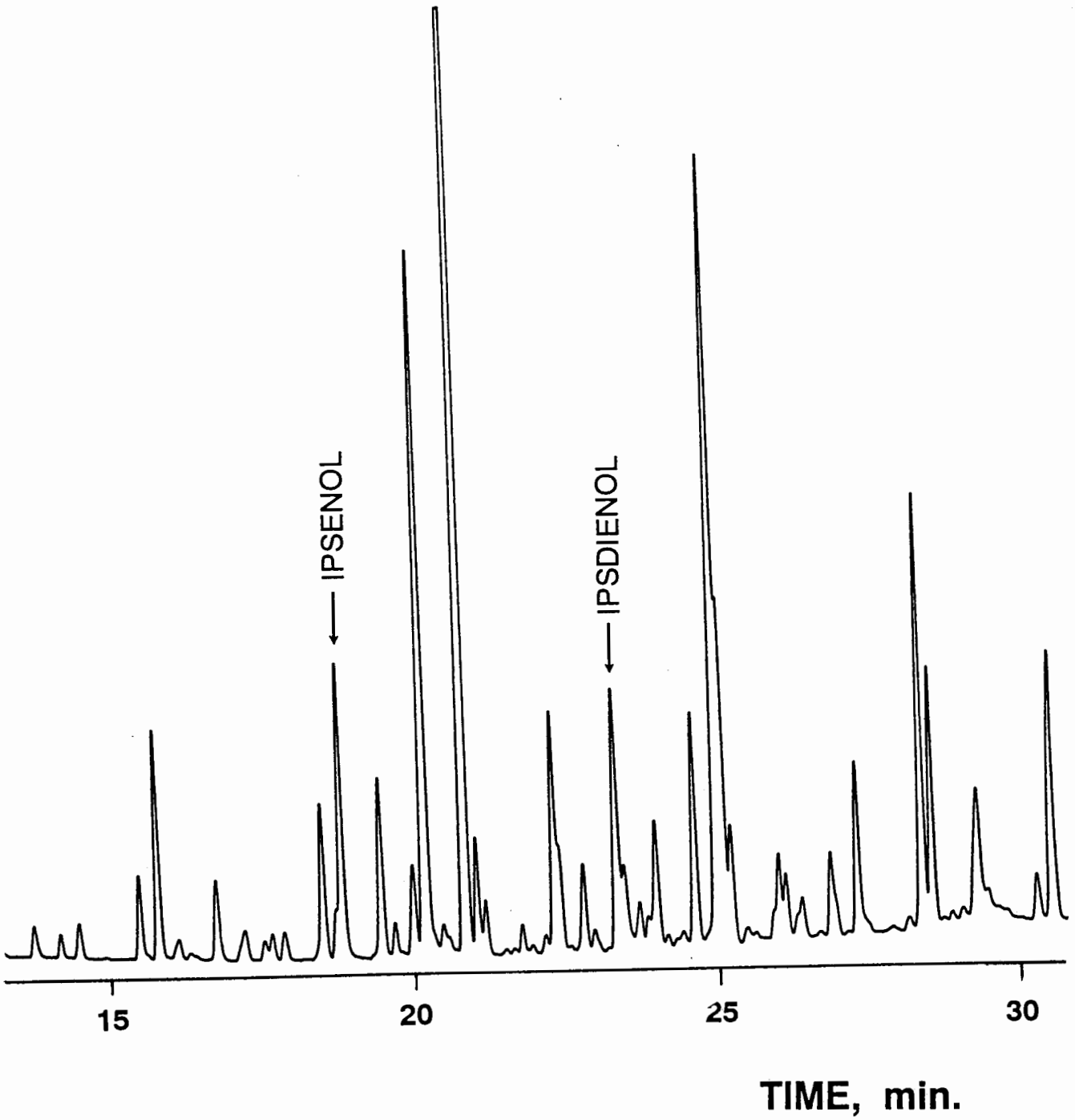
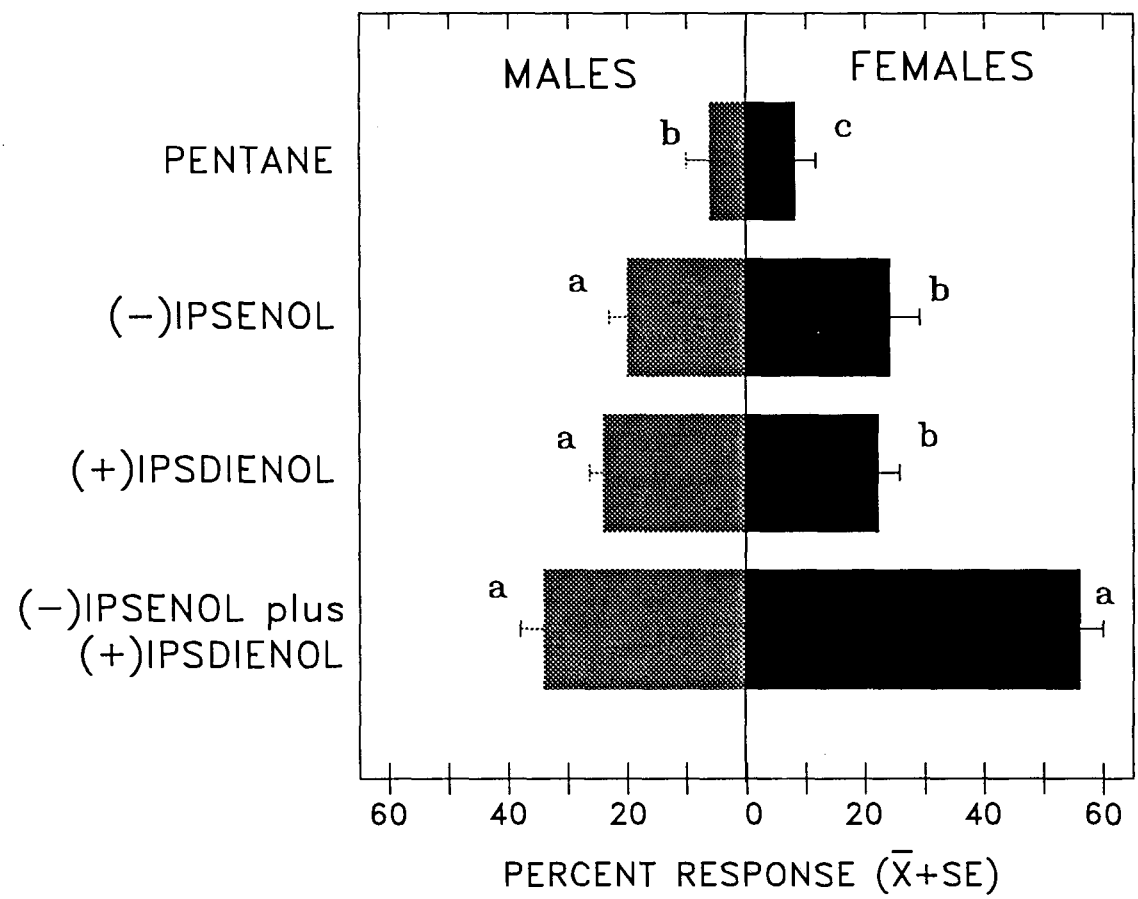

IPSENOL
IPSDIENOL
15
20
25
30
TIME, min.

TABLE 8. Response by *Pityokteines minutus* in laboratory bioassay to fractions of extracts equivalent to 0.6 mg of male-produced frass.

STIMULUS	PERCENT RESPONSE ($\bar{X}$±SE)[a] MALES	FEMALES
PENTANE (10 μL)	6.0±2.4 d	2.0±2.0 d
MALE FRASS EXTRACT	50.0±4.5 ab	68.0±3.7 ab
FRACTION F1	22.0±2.0 c	36.0±5.1 c
FRACTION F2	32.0±4.9 bc	62.0±4.9 ab
FRACTION F3	20.0±3.2 c	10.0±3.2 d
F1 plus F2	24.0±2.4 c	50.0±5.5 bc
F1 plus F3	20.0±3.2 c	12.0±2.0 d
F2 plus F3	42.0±5.8 ab	62.0±5.8 ab
F1 plus F2 plus F3	52.0±6.6 a	72.0±5.8 a

[a]Percents within a column followed by the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$.

Figure 28. Response of *Pityokteines minutus* in laboratory olfactometer bioassays to 1 ng stimuli of (*S*)-(-)-ipsenol, (*S*)-(+)-ipsdienol, and to the combination (1 ng of each). Percents with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$


MALES
FEMALES
PENTANE
(−)IPSENOL
(+)IPSDIENOL
(−)IPSENOL plus
(+)IPSDIENOL
b
c
a
b
a
b
a
a
60
40
20
0
20
40
60
PERCENT RESPONSE (X̄+SE)

Field Experiments. Infested logs containing males were more attractive to *P. minutus* than uninfested logs in Field Exp. 5A, indicating that males produce an attractive pheromone (Table 9). Responses to female-infested bolts and those infested by both sexes were intermediate between those to male-infested and uninfested logs. This result suggests that females alone may induce some aggregation. However, it also indicates that males allowed to mate are not as attractive as single males. Poor catches in this and other field experiments may have been influenced by unusually cold and rainy weather during June through August, 1993.

(-)Ipsenol attracted both male and female *P. minutus* in Field Exp. 5B. (Table 10). (+)Ipsdienol was inactive by itself and did not significantly affect the attractiveness of (-)-ipsenol. Thus (*S*)-(-)-ipsenol is a pheromone component for *P. minutus*. No *Ips* spp. responded to any treatment.

The small numbers of beetles responding may have been caused in part by an incomplete or imprecise chemical signal. Further research with other release rates and ratios of the two compounds tested may disclose results similar to those observed in the laboratory bioassay (Fig. 28). Analysis by coupled gas chromatography-electroantennographic detection (GC-EAD) as can be done for other bark beetles

TABLE 9. Numbers of *Pityokteines minutus* caught in Field Exp. 5A to traps baited with infested and uninfested *Abies lasiocarpa* logs. Six replicates, 6 July to 3 August, and 6 replicates 3 to 31 August, 1993.

	NUMBERS OF BEETLES CAPTURED ($\bar{X}$±SE)[a]	
STIMULUS	MALES	FEMALES
MALE INFESTED LOG	1.1±0.2 a	1.6±0.4 a
FEMALE INFESTED LOG	0.8±0.4 ab	0.8±0.3 ab
MALE AND FEMALE INFESTED LOG	1.2±0.5 ab	1.2±0.7 ab
UNINFESTED LOG	0.2±0.1 b	0.3±0.2 b
UNBAITED	0.2±0.1 b	0.2±0.1 b

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

TABLE 10. Numbers of *Pityokteines minutus* caught in Field Exp. 5B to traps baited with volatile compounds isolated from feeding males. Seven replicates, 6 July to 3 August, and 7 replicates 3 to 31 August, 1993.

	NUMBERS OF BEETLES CAPTURED ($\bar{X}$±SE)[a]	
STIMULUS	MALES	FEMALES
(*S*)-(-)-IPSENOL	0.9±0.4 a	1.4±0.5 a
(*S*)-(+)-IPSDIENOL	0.1±0.1 b	0.1±0.1 b
(*S*)-(-)-IPSENOL plus (*S*)-(+)-IPSDIENOL	0.5±0.2 a	0.9±0.2 ab
UNBAITED	0.1±0.1 b	0.2±0.1 b

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

(Wadhams et al. 1982) could be used to detect additional pheromone components. The response to fractions of male-produced volatiles (Table 8) points out that unidentified attractive compounds may be present in F1 and F3. Two host volatiles abundant in F3 (piperitone and cuminic aldehyde) did not enhance attraction to candidate pheromones when tested in the field (results not shown).

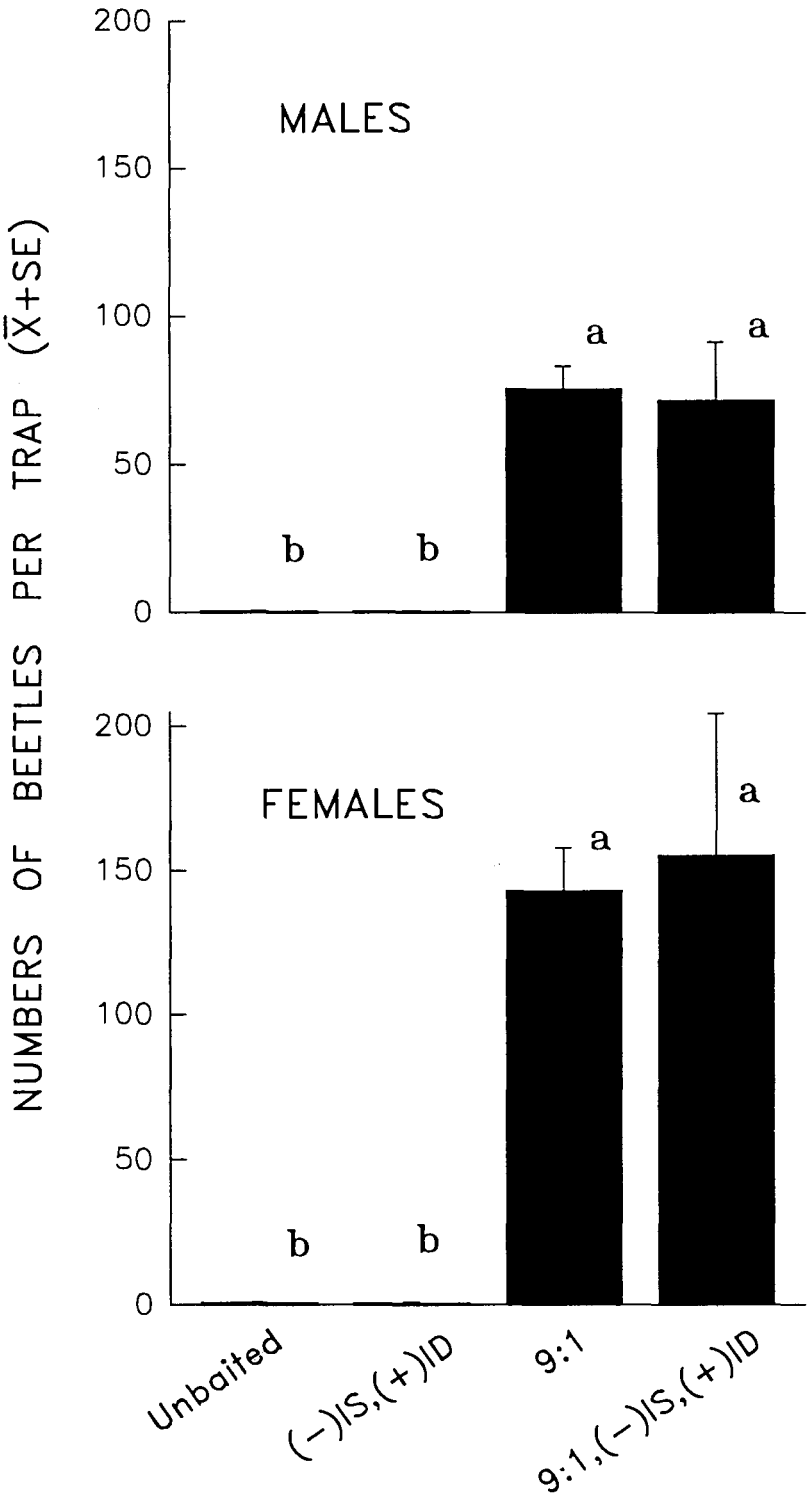
Determination of the presence and activity of (-)-ipsenol in an American *Pityokteines* species is consistent with data from three European congenerics, in which ipsenol and ipsdienol have been identified (Harring et al. 1975; Harring and Mori 1977; Harring 1978). *P. curvidens* was attracted to (-)ipsenol and was inhibited by the two enantiomers of ipsdienol, The sympatric *P. vorontzovi* responded to (±) and (*R*)-(-)-ipsdienol (Harring 1978), which together with my data, suggests a major role of chirality in the response to pheromones within the genus. Enantiospecificity may also be important between sympatric *P. minutus*, *P. elegans* and *Ips* spp.

Personal observations suggest that *P. minutus* has a patchy distribution in the forest. It was either present in abundance or completely absent in similar trees. Two other secondary species present in declining subalpine firs, *Pityophthorus pseudotsugae* Swaine, and *Dryocoetes schelti*,

may have considerable niche overlap with *P. minutus*. The small size (2.0-2.4 mm) and similarities in morphology and in gallery characteristics among these three species make the identification difficult in the field, and made the selection of trapping sites uncertain.

Interspecific Interactions. Responses of *D. confusus* to the 9:1 blend of (+)EXOB:(+)ENDOB were not affected in Field Exp. 5C by the presence of *P. minutus*-produced compounds (Fig. 29). There was no evidence in other field experiments (Section 2) that *P. minutus* is attracted to the 9:1 blend of (+)EXOB:(+)ENDOB. *D. confusus* can respond to host related stimuli (Stock and Borden 1983; Section 3) and typically infests either living or newly dead trees. On the other hand *P. minutus* is found alone in very dry hosts or in debilitated trees already attacked by *D. confusus*. Because neither species is cross-attracted to the other's pheromone, and because *D. confusus* is not repelled by the known components in *P. minutus* volatiles it would appear that the two species co-exist without significant interaction. Thus there is only a slight possibility for the development of semiochemical-induced competitive displacement of *D. confusus* by *P. minutus* as proposed for *D. ponderosae* by *I. pini* (Rankin and Borden 1991).

Figure 29. Numbers of *Dryocoetes confusus* caught in Field Exp. 5C to traps baited with (*S*)-(-)-ipsenol [(-)IS], plus (*S*)-(+)-ipsdienol [(+)ID] alone or combined with the 9:1 blend of (+)EXOB:(+)ENDOB (9:1). Eight replicates 22 July to 31 August, 1993. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$


MALES
FEMALES
NUMBERS OF BEETLES PER TRAP (X̄+SE)
200
150
100
50
0
a
a
b
b
a
a
b
b
Unbaited
(−)IS,(+)ID
9:1
9:1,(−)IS,(+)ID

As for *D. confusus*, the presence of (-)ipsenol and (+)ipsdienol in Field Exp. 5D had no effect on the response of *D. affaber* to its pheromones (Fig. 30). The responses of *P. minutus* to its candidate pheromones were consistent with those in Field Exp. 5B (Table 10), and not significantly affected by the presence of *D. affaber* pheromones (Table 11). However, for both species , there was a numerical trend toward reduction in response in the presence of heterospecific semiochemicals. The two secondary species can be found in the same hosts, and some degree of pheromonal interference could reduce the likelihood of interspecific competition.

Figure 30. Numbers of *Dryocoetes affaber* caught in Field Exp. 5D to traps baited with (*S*)-(-)-ipsenol [(-)IS], plus (*S*)-(+)-ipsdienol [(+)ID] alone or combined with the 1:2 blend of (+)EXOB:(+)ENDOB (1:2). Seven replicates, 6-22 July, and 8 replicates, 22 July to 17 August, 1993. Bars with the same letter are not significantly different, Ryan-Einot-Gabriel-Welsch multiple F test, $P<0.05$

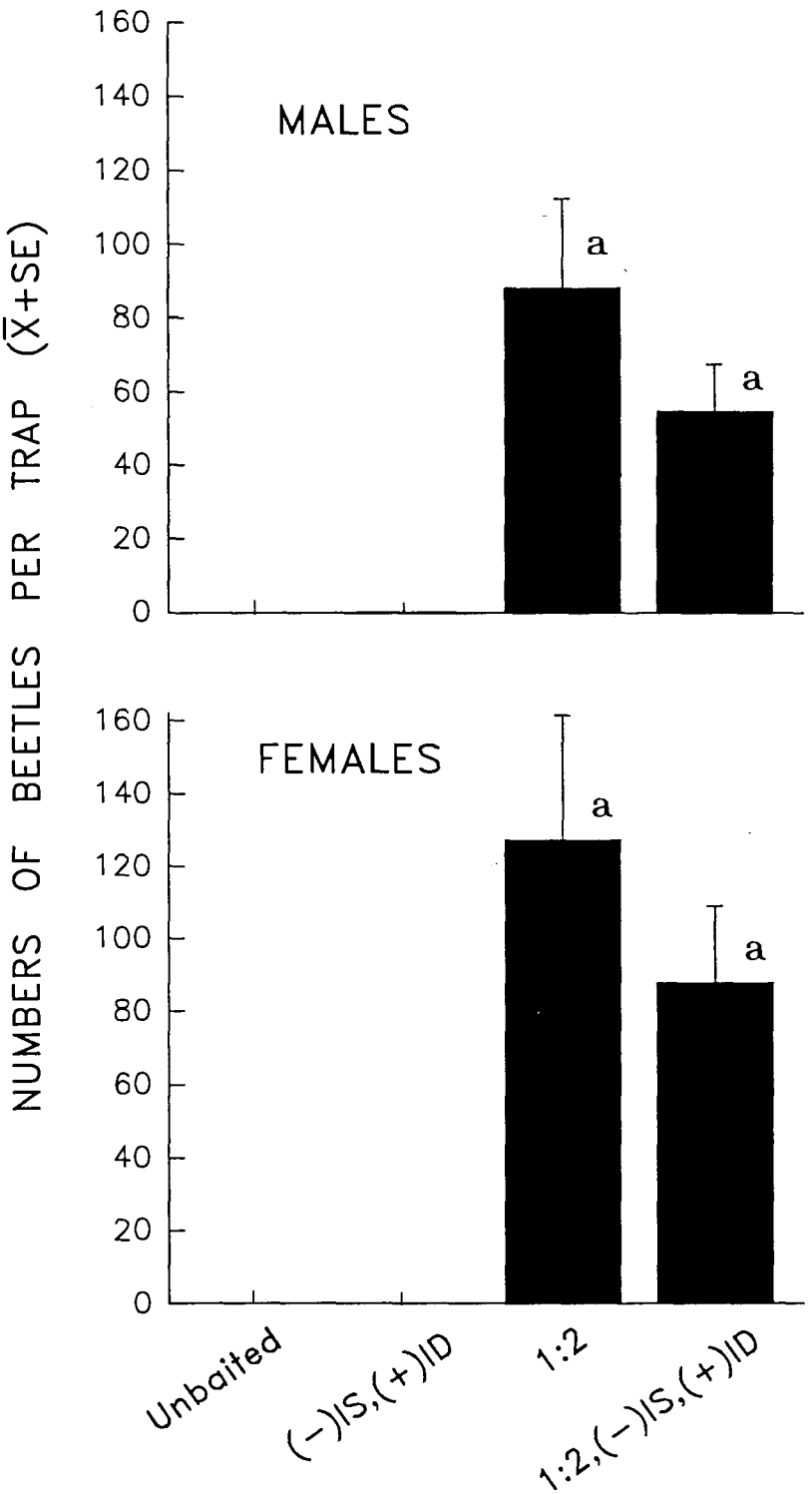

MALES
FEMALES
NUMBERS OF BEETLES PER TRAP (X̄+SE)
160
140
120
100
80
60
40
20
0
a
a
a
a
Unbaited
(−)IS,(+)ID
1:2
1:2,(−)IS,(+)ID

TABLE 11. Numbers of *Pityokteines minutus* caught in Field Exp. 5D to traps baited with (*S*)-(-)-ipsenol plus (*S*)-(+)-ipsdienol alone or combined with the 1:2 blend of (+)EXOB:(+)ENDOB (1:2). Seven replicates, 6-22 July, and 8 replicates 22 July to 17 August, 1993.

	NUMBERS OF BEETLES CAPTURED ($\bar{X}$±SE)[a]	
STIMULUS	MALES	FEMALES
(-)IPSENOL, (+)IPSDIENOL	0.7±0.4 a	2.3±1.1 a
1:2, (-)IPSENOL, (+)IPSDIENOL	0.5±0.2 a	0.9±0.2 a
1:2	0.1±0.1 b	0.1±0.1 b
UNBAITED	0.1±0.1 b	0

[a]Means followed by the same letter are not significantly different, Friedman test, $P<0.05$. Treatments with zero catches not included in analysis.

6. SUMMARY AND CONCLUSIONS

I conducted research on the chemical communication system of the western balsam bark beetle, *Dryocoetes confusus*, and two secondary scolytid species, *D. affaber* and *Pityokteines minutus*; all of them are sympatric in the subalpine forests of B.C. The results of these studies are summarized in Table 12. For *Dryocoetes confusus* and *D. affaber*, my results indicate that the combined effect of optical and geometrical isomerism of brevicomin determines both the level of response and the species-specificity of the pheromones. The same compounds are pheromones in European *D. autographus* (Kohnle and Vité 1984), and possibly in *D. hectographus* (Miller et al. 1989). Thus, it appears that the stereoisomers of brevicomin constitute the basis of the chemical communication in the genus. The presence in B.C. of *D. autographus, D. schelti,* and *D. caryi* (Bright 1963) provides an opportunity for further study of the mechanisms of pheromonal specificity.

The practical importance of chirality was demonstrated by the significantly higher response of *D. confusus* to enantiospecific baits on trees than to racemic baits (Fig. 9,

TABLE 12. Summary of results on semiochemicals for three species of bark beetles in subalpine forests of B.C..

STUDIED	***DRYOCOETES CONFUSUS***	***DRYOCOETES AFFABER***	***PITYOKTEINES MINUTUS***
PHEROMONES PRODUCED	EXOB : ENDOB	EXOB : ENDOB	IPSENOL:IPSDIENOL
Chirality	+ : +	+ : +	- : +
Whole body extract	18 : 1	1.7 : 1	
Frass	5 : 1		1.1 : 1
Aeration	11 : 1	3 : 1	1.3 : 1
LABORATORY BIOASSAY			
Best response to	9 : 1	1 : 2	1 : 1 [a]
	+ : +	+ : +	- : +
FIELD EXPERIMENTS			
Best response to	9 : 1	1 : 2	(-)IPSENOL
	+ : +	+ : +	
	or + : ±	or ± : +	
HOST TREE KAIROMONES	(-)-α-PINENE		
	(R)-(-)-MYRTENAL		
REMARKS	(-)EXOB may inhibit males	(-)ENDOB may be inhibitory	Missing components ?

[a] Other ratios not tested

Table 5). Progress in the field of organic synthesis, e.g. enzymatic syntheses (Ramaswamy and Oehlschlager 1989), may provide cost-effective methods for producing optically pure compounds, which may improve the effectiveness, and promote the use of chiral semiochemicals in pest management.

The systematic isolation and identification of plant kairomones yielded two compounds (-)-α-pinene and (*R*)-(-)-myrtenal, that significantly enhanced the response of *D. confusus* to traps baited with specific pheromones (Fig. 16). The relatively low cost of the kairomones, and the availability of adequate formulations, and controlled-release technology suggest that they could be incorporated into semiochemical blends used in the management of *D. confusus*.

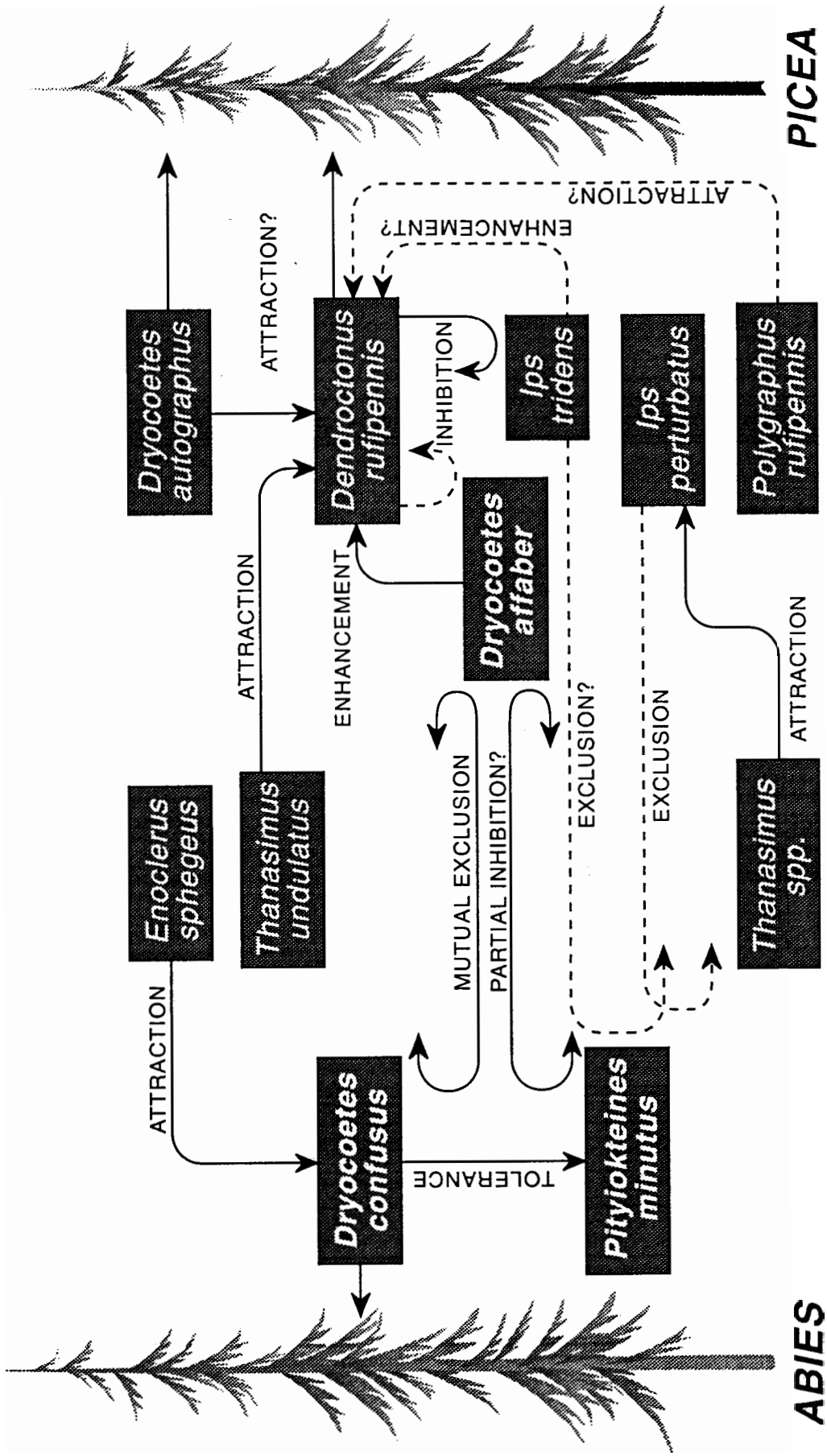
Laboratory and field experiments provided evidence of secondary attraction in *P. minutus*. Classical isolation and identification procedures (Silverstein et al. 1967) disclosed production and response to (-)ipsenol and (+)ipsdienol, but field results indicated that (+)ipsdienol was inactive (Table 10). Further research on the chirality, ratio and release rates of these two compounds, and on the search for additional semiochemicals for *P. minutus*, is required to provide a complete understanding of its semiochemical-based communication. Application of gas chromatography-electroantennographic detection (Cork et al.

1990; Hallett et al. 1993) may facilitate this understanding.

The general systems theory (von Bertalanffy 1928, 1950, 1968) postulates that the study of isolated parts and processes does not result in a complete explanation of functional systems. Such investigations do not provide information about the coordination of parts and processes, nor do they elucidate the interactions among elements that may result in "emergent properties" characteristic of the new level of organization and inherent to a particular state of the system.

My results provide information on some of the species present in the subalpine forest, and on some of their interactions at the semiochemical level. Integration of this information with results from other studies, allows an emerging picture of the system to be visualized (Figure 31). *D. confusus* and *D. affaber* show mutual pheromonal exclusion (Fig. 25). Response of *D. confusus* is not affected by the presence of (-)ipsenol and (+)ipsdienol, compounds produced by male *P. minutus* (Fig. 29). The reciprocal responses of *D. affaber* and *P. minutus*, although not significantly different, appear to show some level of mutual inhibition (Fig. 30).

Figure 31. Schematic model of semiochemical-based interactions at three trophic levels between some bark beetles and associated insects present in the subalpine forest of B.C. and NW North America. Solid arrows represent direct experimental evidence, broken ones preliminary or indirect evidence.


PICEA
ABIES
Dryocoetes autographus
Dendroctonus rufipennis
Ips tridens
Ips perturbatus
Polygraphus rufipennis
Dryocoetes affaber
Enoclerus sphegeus
Thanasimus undulatus
Thanasimus spp.
Dryocoetes confusus
Pityiokteines minutus
ATTRACTION?
ENHANCEMENT?
ATTRACTION?
INHIBITION
ATTRACTION
ENHANCEMENT
MUTUAL EXCLUSION
PARTIAL INHIBITION?
EXCLUSION?
EXCLUSION
ATTRACTION
ATTRACTION
TOLERANCE

Borden et al. (1987), reported attraction of the clerid *Enoclerus sphegeus* (F.) to (±)EXOB, deployed as an attractant for *D. confusus*. The virtual absence of *Ips* spp. in traps baited with (-)ipsenol alone or together with (+)ipsdienol, and data from Alaska indicating the response of *I. perturbatus* to (±) and (-) ipsdienol (Werner 1993), lead me to hypothesize a mechanism of pheromonal specificity based on the chirality and ratios of ipsenol and ipsdienol in *I. tridens, I. perturbatus* and *P. minutus*.

Clerid predators *Thanasimus* spp. responded to ipsenol deployed as bait for *I. perturbatus* (Werner 1993) and *Thanasimus undulatus* Say, was attracted to traps baited with fontalin, a pheromone of the spruce beetle, *Dendroctonus rufipennis* (Kline et al. 1974). *Dryocoetes autographus* was also collected in traps containing attractants for the spruce beetle (Furniss et al. 1976). Interestingly very few *D. autographus* were found in traps baited with pheromones for *D. confusus* and *D. affaber*.

Preliminary results from field experiments conducted by T.M. Poland (pers. comm.[1]), indicate an enhanced response from *D. affaber* to traps baited with its specific pheromones

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[1] Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.

plus (±)-frontalin and α-pinene, a commercial bait for the spruce beetle (Phero Tech Inc., Delta, B.C.). Conversely, the response of spruce beetle to its semiochemicals, was reduced in the presence of *D. affaber* pheromones. A similar effect was observed with the combination of spruce beetle bait, plus (-)-*cis*-verbenol and (+)ipsdienol, hypothesized aggregation pheromones of *I. tridens* (Moeck et al. 1985), which like *D. affaber*, showed a trend towards increased response to the combined baits. Furniss et al. (1976), reported catches of *Polygraphus rufipennis* (Kirby) in traps baited with an uninfested spruce log, plus frontalin, and/or *trans*-verbenol, deployed as candidate attractants for the spruce beetle. The semiochemicals employed by other elements of the system (including several secondary bark beetles, species of Buprestidae and Cerambycidae, as well as entomophagous insects), have not been studied.

The role of the host tree, has also received little attention, with exception of my results and those of Borden et al. (1987) on the interaction of host monotepenes with pheromones of *D. confusus*, the role of the ethanol in inducing attack by spruce beetles (Moeck 1981), the inclusion of α-pinene in commercial baits for spruce beetles, and the response of *D. autographus* to the combination of monoterpenes and ethanol (Chénier and Philogène 1989). It is probable that host recognition

precedes pheromone production by some species and regulates response specificity by others.

It is clear that the semiochemical-based communication system for bark beetles in subalpine forests of B.C. is far from being completely elucidated. However, the emerging picture (Fig. 31) includes three trophic levels, focused around the interactions of several scolytid species. These involve various combinations of intraspecific messages, interlaced with interspecific messages that mediate cross or enhanced attraction, tolerance, and mutual or unilateral exclusion, which would depend on the adaptive advantage imparted. All of these may be mediated in some way by host tree volatiles, as may be the response of generalist or species-specific parasites and predators to their hosts.

Although the prospects for semiochemical-based management of *D. confusus* based on current knowledge appear good (Stock et al. 1993; Fig. 9), careful analysis of this emerging system (Borden 1989) may disclose more avenues for semiochemical-based manipulation of the major pest species, *D. rufipennis* and *D. confusus*.

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