REPRODUCTIVE AND ECOLOGICAL ISOLATION: COMMUNITY STRUCTURE IN THE USE OF SEMIOCHEMICALS BY PINE BARK BEETLES (COLEOPTERA: SCOLYTIDAE)

by

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Reproductive and ecological isolation: Community structure in the use of semiochemicals by pine bark beetles (Coleoptera: Scolytidae)

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Abstract

I studied the use of semiochemicals by pine bark beetles (Coleoptera: Scolytidae) with respect to patterns of species-specificity and geographic variation; patterns which are often associated with reproductive and ecological isolation. Seasonal variation in flight periods contributes to reproductive isolation among the three sympatric species, *Ips latidens*, *I. pini* and *Dendroctonus ponderosae*. However, the separation may not be constant between years. Dose-dependent and all-or-none responses to kairomones, pheromones and synomones, by the three species, also act in concert to maintain species-specificity. *Dendroctonus ponderosae* differs from *I. latidens* and *I. pini* in using γ-terpinene and myrcene as attractive kairomones, while *D. ponderosae* and *I. pini* differ from *I. latidens* by using 3-carene. The use of β-pinene by *I. pini* differentiates it from *D. ponderosae*. All three species are attracted to β-phellandrene. The pheromones for *I. latidens* and *I. pini* are ipsenol and ipsdienol, respectively, while *D. ponderosae* uses *cis*- and *trans*-verbenol in conjunction with *exo*-brevicomin. The use of ipsenol by *I. latidens* and *cis*-verbenol by *D. ponderosae* are new discoveries. All of the pheromones acted as mutually-inhibitory synomones, except that ipsenol had no effect on *D. ponderosae*. Inter- and intrapopulation variation in the production of chiral ipsdienol was found to occur between populations of *I. pini* in British Columbia as well as between populations from New York and California. There was a lack of enantio-specificity in responses of *I. pini* to ipsdienol in four populations in British Columbia. I hypothesise that post-glaciation colonisation, and subsequent reproductive and ecological isolation in the western United States, are responsible for the geographic pattern in the use of chiral ipsdienol as a pheromone by *I. pini* in North America. I conclude that patterns of species-specificity and geographic variation occur in the use of semiochemicals by bark beetles. The causative factors may include random drift, post-glaciation colonisation, interspecific reproductive interference and interspecific competition.

Dedicated to the memory of Gerry Lanier

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J. Gandy, R. Gries, C. Matteau, C. Pon, T. Richerson and L. Wheeler assisted in processing catches. Identifications of *Ips pini*, *I. latidens* and *Dendroctonus ponderosae* were kindly verified by D.E. Bright. Assistance in the manufacture of release devices was provided by PheroTech Inc. This research was supported by an H.R. Macmillan Family Fund Fellowship, a Simon Fraser University Graduate Research Fellowship, a Simon Fraser University President's Research Stipend, Grant No. 1 (RC 14-16) from the Science Council of British Columbia, and Operating and Strategic Grants A0851, A3785, A3881 and G1039 from the Natural Sciences and Engineering Research Council of Canada.

I thank H.G. Merriam for his infectious zeal for ecology; he provided my initial motivation to become an ecologist. And lastly, I extend my gratitude and heartfelt thanks to members of my family, and numerous friends, for providing much needed support and diversions.

Preface

I followed three conventions in this thesis for the sake of clarity. Firstly, I used a narrow-sense definition of communication (Burghardt 1970). I suggest that semiochemical-based information-transfer systems can be divided into systems involving communication (pheromones and synomones) and systems involving unilateral exploitation of information (kairomones and allomones). The selection pressures involved in the use of pheromones should be more similar to those involved in the use of synomones than to those involved in the use of kairomones and allomones. Secondly, I omitted authors and taxonomic positions of tree and insect species from the text and grouped them, instead, into Tables 13 and 14, respectively, in the Appendix. Thirdly, I only used common names of scolytid pheromones and synomones in the text and listed their corresponding IUPAC names in Table 15 in the Appendix. Chapters 2.2.1, 2.2.2 and 3.1.1 have been previously published, in part, as follows:

Chap. 2.2.1 Miller, D.R. and J.H. Borden. 1990. The use of monoterpenes as kairomones by *Ips latidens* (LeConte) (Coleoptera: Scolytidae). *Can. Ent.* **112**: 301-307.

Chap. 2.2.2 Miller, D.R. and J.H. Borden. 1990. β-Phellandrene: Kairomone for the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). *J. Chem. Ecol.* **16**: 2519-2531.

Chap. 3.1.1 Miller, D.R., J.H. Borden and K.N. Slessor. 1989. Inter- and intrapopulation variation of the pheromone, ipsdienol, produced by male pine engravers, *Ips pini* (Say) (Coleoptera: Scolytidae). *J. Chem. Ecol.* **15**: 233-247.

Borden, J.H., D.W.A. Hunt, D.R. Miller and K.N. Slessor. 1986. Orientation in forest Coleoptera: An uncertain outcome of responses by individual beetles to variable stimuli. pp 97-109, in T.L. Payne, M.C. Birch and C.E.J. Kennedy (eds.). Mechanisms in insect olfaction. Oxford University Press.

Slessor, K.N., G.G.S. King, D.R. Miller, M.L. Winston and T.L. Cutforth. 1985. Determination of chirality of alcohol or latent alcohol semiochemicals in individual insects. *J. Chem. Ecol.* **11**: 1659-1667.

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1 INTRODUCTION

1.1 COMMUNITY PATTERNS IN THE USE OF SEMIOCHEMICALS

In ecology, as in many other disciplines, we look for patterns that deviate from random or expected and then attempt to understand why such deviations exist. The existence of patterns usually implies some sort of selection process (directed or random). These patterns might involve such relationships as the size and shape of bird beaks relative to the size and shape of food items, the distribution of plant species relative to altitude, or the specificity in chemical signals within a community of insects. My thesis concentrates on the latter, focusing on the use of semiochemicals (= infochemicals) (Law and Regnier 1971; Nordlund and Lewis 1976; Nordlund 1981; Dicke and Sabelis 1988) within communities of bark beetles (Coleoptera: Scolytidae).

Two general types of community patterns, typical of many systems, are evident with respect to the use of semiochemicals: species-specificity and intraspecific geographic variation. In the first type, some semiochemical character is partitioned among species within a community, resulting in species-specificity for that character. Species-specificity may serve as a mechanism for reproductive and ecological isolation (Fisher 1930; Mayr 1959; Brown and Wilson 1956; Wood 1970; Grant 1972; Lanier and Burkholder 1974; Roelofs and Cardé 1974; Shorey 1977; Matthews and Matthews 1978; Roelofs and Brown 1982; Cardé and Baker 1984; West-Eberhard 1984; Cardé 1986; Linn and Roelofs 1989). In Wisconsin, reproductive isolation is maintained among 29 species of clear-winged moths (Sessiidae) by partitioning pheromone channels along three dimensions: chemical composition, and seasonal and diel activity (Greenfield and Karandinos 1980). Individuals of sympatric species of small ermine moths (Yponomeutidae) use pheromone composition, host-kairomone specificity and temporal variation to maintain reproductive isolation (Hendrikse 1979; van der Pers 1981: Löfstedt et al. 1986). The chemical

composition of cephalic marking secretions, used by male bumble bees to mark objects within their territories, is species specific among 19 species of *Bombus* and *Psithyrus* (Hymenoptera: Apidae) (Kullenberg et al. 1970). Among seven species of tortricid moths (Lepidoptera: Tortricidae), partitioning occurs along one dimension: the *E:Z* ratio of 11-tetradecenyl acetate (Roelofs et al. 1974; Cardé et al. 1977; Roelofs and Brown 1982).

Evidence of similar patterns is apparent in the use of aggregation pheromones by bark and ambrosia beetles. Interspecific inhibition of response to pheromones occurs among various pairs of bark beetle species in western North America such as between *Ips paraconfusus* and *I. pini* (Birch and Wood 1975; Birch and Light 1977; Birch et al. 1977; Birch 1978; Light and Birch 1977; Birch et al. 1980a), and between *Dendroctonus ponderosae* and *I. paraconfusus* (Byers and Wood 1980, 1981). In Europe, reproductive isolation among six *Ips* species seems to be facilitated by species-specificity in pheromone composition and mutual inhibition of responses (Kohnle et al. 1986, 1988). In the southern United States, four sympatric species of bark beetles breed in loblolly pine, often with three or all four present in the same tree (Birch 1978; Dixon and Payne 1979; Birch and Svihra 1979; Svihra et al. 1980; Paine et al. 1981; Byers 1989a). Reproductive isolation seems to be facilitated by partitioning of the use of pheromones (Vité and Francke 1976; Birch 1978; Vité et al. 1978; Birch et al. 1980b; Svihra et al. 1980). In British Columbia three species of ambrosia beetles commonly attack logs in dryland timber sorting areas (Nijholt 1978). Female *Trypodendron lineatum* produce lineatin as a sex pheromone (MacConnell et al. 1977; Borden et al. 1980b; Slessor et al. 1980), while *Gnathotrichus sulcatus* and *G. retusus* use sulcatol with chiral ratios of 65:35 and 100:0 (+):(-), respectively (Byrne et al. 1974; Borden et al. 1976; Borden et al. 1980a). The three species show preference for their respective pheromones (Borden and McLean 1979,1981; Borden et al. 1981).

However, cross-attraction between species of bark beetles does occur. In the southern United States, *Ips avulsus* is sympatric with *I. grandicollis* and *I. calligraphus*;

all three breed in loblolly pine (Furniss and Carolin 1980). *Ips avulsus* is attracted to the pheromone produced by male *I. calligraphus* (Birch et al. 1980b; Svihra et al. 1980). Attraction of *I. avulsus* to conspecifics is enhanced by actively-boring *I. grandicollis*. *Ips grandicollis*, on the other hand, is attracted to boring female *D. frontalis*.

An apparent conflict, therefore, appears to exist between the phenomena of species-specificity and cross-attraction with respect to the use of pheromones in maintaining reproductive and ecological isolation within communities of bark beetles (Lanier and Burkholder 1974; West-Eberhard 1984). This conflict arises because bark beetle pheromones convey two types of information to both con- and heterospecifics: mating opportunities and the availability of suitable host material. Heterospecifics should obviously avoid responding to such pheromones with respect to mating opportunities. However such individuals might benefit in responding to the information regarding host availability by moving closer to the source (Birch et al. 1980b). Many species of bark beetles show some degree of resource partitioning, subdividing a tree, for instance, into discrete areas: roots, stump, lower and upper boles, large and small branches, and cones. Bark beetles should be attracted to the pheromone of heterospecifics if they occupy separate areas on the same host, thereby minimising the risk of predation as well as the expenditure of time and energy.

However, interspecific attraction should still decrease with increasing proximity to the sites of production; reproductive isolation must still be ensured. The use of additional information from other semiochemicals, or from visual or acoustic cues could provide this assurance. Sound production by female bark beetles is clearly species-specific (Barr 1969; Lanier 1970). The apparent lack of observed species-specificity in semiochemical-based communication may be a consequence of measuring responses at inappropriate points in the series of decisions that scolytid beetles must make in the process of host selection (D.L. Wood 1982; Borden 1982).

The second type of community pattern in the use of semiochemicals concerns variation of a character between geographically-separate populations of the same species (Roelofs 1980; Cardé and Baker 1984). The European corn borer, *Ostrinia nubilalis*, uses 11-tetradecenyl acetate (11-14:OAc) as a sex pheromone (Klun 1968; Klun and Brindley 1970; Klun and Robinson 1971). Geographic variation is based on the relative proportion of the *E* and *Z* isomers of 11-14:OAc (Klun et al. 1973; Kochansky et al. 1975). Most populations in Europe and North America respond to a 3:97 *E:Z* ratio of 11-14:OAc while populations in Italy, the Netherlands and parts of northeastern United States respond preferentially to a 97:3 *E:Z* blend (Klun and Cooperators 1975). Some areas, such as New York state, have discrete populations using only the 3:97 *E:Z* blend as well as hybrid populations (Roelofs et al. 1985). In California, at least three different populations of the western avocado leafroller, *Amorbia cuneana*, differ in the production of *E:Z* blends of the sex pheromone: *(E,E/Z)*-10,12-tetradecadien-1-ol acetate. Two populations respond to blends close to 1:1 while the third prefers blends with a higher *E:Z* ratio (Bailey et al. 1986). In New Zealand, brownheaded leafrollers, *Ctenopseustis obliquana*, exhibit two different pheromone population types; one uses an 80:20 mix of *(Z)*-8-tetradecenyl and *(Z)*-5-tetradecenyl acetates while the other uses only *(Z)*-5-tetradecenyl acetate (Foster and Roelofs 1987).

Evidence of geographic variation in the use of semiochemicals is not abundant in bark beetles. The clearest example concerns the use of the pheromone, ipsdienol, by the pine engraver, *Ips pini* (Lanier 1972; Birch 1978). Males from California (Stewart 1975; Birch et al. 1980a), Idaho (Plummer et al. 1976) and southeastern British Columbia (Slessor et al. 1985; Borden et al. 1986) produce primarily (-)-ipsdienol while males from New York produce a 65:35 mixture of the (+) and (-) enantiomers (Lanier et al. 1980). In California, *I. pini* are attracted to sources of (-)-ipsdienol but repelled by (+)-ipsdienol (Birch et al. 1980a). In New York, beetles respond best to racemic ipsdienol (Lanier et al. 1980).

Reproductive isolation and competition are often cited as the selective pressure behind these patterns (Cardé and Baker 1984; Cardé 1986). Species-specificity in the use of a semiochemical character can ensure niche separation and mating specificity. Character displacement should be a consequence of species abundance, or relative abilities of different species to compete for a resource (Brown and Wilson 1956; Grant 1972; Eldredge 1974). If species numbers or species composition vary between geographically-separate locations then selection pressures should also vary, resulting in variation in character displacement for any given species found at both locations. Other pressures may include mutation as well as stabilising selection and sexual selection (Baker and Cardé 1979; Cardé and Baker 1984; West-Eberhard 1984; Cardé 1986; Löfstedt 1990).

Documentation of such patterns in the use of semiochemicals should further our understanding of the phenomenon of communication in general. Further, such understanding may have implications for pest management programs. Semiochemicals are gaining prominence in efforts to control pest populations. How great is the risk of the development of "resistance" by the target species (Roelofs and Comeau 1969; Lanier et al. 1972; Price 1981; Haynes et al. 1984; Haynes and Baker 1988)? The development of "resistance" has been shown with laboratory populations of the khapra beetle, *Trogoderma granarium*. There was a 74 % reduction in mean response by males to the pheromone produced by wild-type female beetles after 18 generations of selection for nonresponse by males (Rahalkar et al. 1985). The existence of geographically-separate races using different pheromone blends strongly implies that "resistance" is possible in the field as well (Lanier et al. 1972). Determination of the magnitude and significance of the risk requires estimates of variation and heritability (Lanier and Burkholder 1974; Sturgeon and Mitton 1982; Cardé and Baker 1984; Haynes et al. 1984; Collins and Cardé 1985; Haynes and Baker 1988).

The dynamics of species-specificity within a community may be important for pest management as well. How dependent are the species on heterospecific transfer of information? Is there a possibility that the use of a semiochemical against one pest species inadvertently facilitates a previously nondeleterious species in gaining pest status? The use of the antiaggregation pheromones, verbenone and *exo*- and *endo*-brevicomins, to protect loblolly pines from attack by the southern pine beetle, *D. frontalis*, resulted in a 17-fold increase in attack density by *I. avulsus*; all but one of the treated trees died (Watterson et al. 1982).

1.2 THESIS OBJECTIVES

My objectives were twofold. The first was to describe the separation of the use of semiochemicals among three sympatric species of bark beetles, paying particular attention to three classes of semiochemicals as defined by Nordlund (1981): 1) kairomones (as indicators of host quality); 2) pheromones (as indicators of mating opportunities, host quality, and levels of intraspecific competition); and 3) synomones (as indicators of host quality and levels of interspecific competition). *Dendroctonus ponderosae*, *I. pini* and *I. latidens* are broadly sympatric in British Columbia and northwestern United States (Bright 1976; Furniss and Carolin 1980; S.L. Wood 1982). All three commonly breed in lodgepole pine, and often share the same host. *Dendroctonus ponderosae* is an aggressive species, attacking and killing mature pines, and earning the title of "the most damaging insect pest of lodgepole pine" (Furniss and Carolin 1980). The two other species, *I. pini* and *I. latidens*, are secondary pests of lodgepole pine, generally breeding in slash material or the tops and branches of trees killed by *D. ponderosae* (Furniss and Carolin 1980).

Considerable information already exists regarding the use of semiochemicals by *D. ponderosae*; considerably less is known about semiochemicals used by *I. pini* and *I.*

latidens (Borden 1982). I therefore planned to identify critical semiochemicals (particularly for *I. pini* and *I. latidens*) that are used as kairomones, pheromones or synomones by the three species, and then to quantify the effect of interactions between semiochemicals on behavioral responses.

The second objective was to describe the geographic variation in the production of, and response to, chiral ipsdienol by the pine engraver, *I. pini*, paying particular attention to both inter- and intrapopulation variation. *Ips pini* is transcontinental in its distribution, ranging from Newfoundland to British Columbia, Canada to Mexico (Bright 1976; S.L. Wood 1982). Host types include various species of pine and spruce. As a consequence of different localities and different hosts, *I. pini* breeds in communities of different assemblages of scolytid species (Bright 1976; Furniss and Carolin 1980; S.L. Wood 1982), thereby presumably encountering variation in competitive pressures. If competition does structure the use of semiochemicals in a community then we should expect variation to be correlated with levels of competition such as reduced numbers of species and/or relative competitive abilities of different species. Measures of intrapopulation variation should also help address the question of "resistance" to semiochemical-based pest management tactics.

2 MECHANISMS OF ISOLATION AMONG THREE SYMPATRIC SPECIES OF BARK BEETLES

2.1 TEMPORAL SEPARATION

Reproductive isolation among different species in a community can be attained by temporal separation (diel and seasonal) of pheromone-related activities among individuals of different species (Roelofs and Cardé 1971, 1974; Brown 1972; Shorey 1974; Silberglied 1977; Teal et al. 1978; Hendrikse 1979; Cardé and Baker 1984; Cardé 1986). Two sympatric species of plume moths, *Platyptila carduidactyla* and *P. williamsii*, use the same sex pheromone: *(Z)*-11-hexadecenal. Reproductive isolation between these two species is achieved through diel separation of pheromone-related activities; *P. carduidactyla* females call and males respond during the first half of scotophase while *P. williamsii* communicates during the second half (Haynes and Birch 1986). Seasonal separation of pheromone-related activity is a critical component in maintaining reproductive isolation among species of Sessiidae (Lepidoptera) (Greenfield and Karandinos 1979). However for most species, diel and seasonal variation are usually insufficient to ensure reproductive isolation, and are generally important only in conjunction with other isolating mechanisms (Sanders 1971; Roelofs and Cardé 1971, 1974; Kaae et al. 1973; Liebherr and Roelofs 1975; Tamaki and Yamaya 1976; Cardé et al. 1977; Grant 1977).

2.1.1 Seasonal separation of semiochemical-mediated flight periods of *Ips latidens*, *I. pini* and *Dendroctonus ponderosae*

2.1.1.1 Introduction

Bark beetles exhibit seasonal separation of flight activities (Dixon and Payne 1979; Furniss and Carolin 1980). Most of a bark beetle's life is spent under the bark. Beetles lay eggs, feed as larvae, pupate and then feed as teneral adults in the phloem tissue of trees (Stark 1982). Once maturation is completed, adults bore through the bark and fly off to another host to breed. Eggs from one female are laid within a short period of time, usually at the same time as those of females in adjacent territories. The result is a synchrony of development, maturation, and ultimately flight of newly-emerged beetles. Synchrony of flight for many scolytids may also occur as a consequence of an overwintering period. Whether they overwinter in trees, as does *D. ponderosae* (Safranyik et al. 1974), or in the forest floor (duff), as does *Ips pini* (Livingston 1979), conspecifics tend to fly to new hosts within a short period of time relative to the entire season.

In British Columbia, *D. ponderosae* generally overwinter as third instar larvae and have a single flight in mid-summer to early fall (Safranyik et al. 1974; McMullen et al. 1986). My preliminary data suggested that *I. pini* in southwestern British Columbia has two major flight periods. The first occurs in early spring and consists of overwintered adults. The second occurs in late summer and consists of first-generation beetles. *Ips latidens* has a single flight period occurring from early to late summer (Furniss and Carolin 1980; personal observation). *Ips latidens* and *D. ponderosae* have only one generation per year (Safranyik et al. 1974; Furniss and Carolin 1980; Miller and Borden 1985; McMullen et al. 1986), while *I. pini* may have 2-3 generations per year in southwestern BC (Reid 1955; Livingston 1979; Furniss and Carolin 1980; personal observation). Since very little is known regarding diel separation of flight periods of bark

beetles, presumably due to a lack of such separation, I focused my efforts only on estimating the degree of seasonal separation in flight periods among *D. ponderosae*, *I. latidens* and *I. pini*. Specifically, I hypothesized that *I. latidens*, *I. pini* and *D. ponderosae* have some degree of seasonal separation of their pheromone-mediated flight periods in stands of lodgepole pine in southwestern British Columbia.

2.1.1.2 Materials and Methods

Species-specific semiochemical lures (see Chaps. 2.2.3, 2.3.1, 2.3.2, and 2.3.3) for *I. latidens*, *I. pini* and *D. ponderosae* were obtained from PheroTech Inc. (Delta BC). The lure for *D. ponderosae* consisted of a closed, polyethylene screw-cap bottle (15 mL) filled with β-myrcene (chemical purity, 98%), a polyethylene bubble-cap containing a 13:87 mix of *cis*- and *trans*-verbenol (chemical purities, 98% for both; chiral ratios, 83:17 (-):(+) for both), and a laminar lure (6.45 cm^2) containing *exo*-brevicomin (chemical purity, 98%). The release rates of these three compounds were approximately 281.3, 2.6, and 0.1 mg/day, respectively, at 24 °C. The lures for *I. latidens* and *I. pini* consisted of bubble-caps containing 3-butanediol solutions of racemic ipsenol and racemic ipsdienol, respectively (chemical purities 98% for both). The release rates for each of these two chemicals was approximately 0.2-0.3 mg/day at 24 °C.

Five randomised blocks (replicates), consisting of three 12-unit multiple-funnel traps (Lindgren 1983), per block, were set linearly in mature stands of lodgepole pine near Princeton BC, in 1989. The three treatments in each replicate were the separate species-specific lures. Three replicates were placed in the Shinnish Creek watershed, approximately 38 km northeast of Princeton, on 24 Apr, 24 Apr, and 31 May, respectively. Two replicates were placed in the Spukune Creek watershed, approximately 28 km northeast of Princeton, on 31 May. Replicates were placed at least 200 m apart, and traps spaced 20 m apart within a replicate. Each trap was suspended

between trees by rope such that the top funnel of each trap was 1.3-1.5 m above ground. No trap was within 2 m of any tree. All lures were replaced on 25 July and the experiment terminated on 26 Sept. Trap catches were collected approximately once per week. Sexes of captured *I. pini* were determined using declivital characters (Lanier and Cameron 1969), while those of captured *I. latidens* and *D. ponderosae* were determined by dissection and internal examination of genitalia.

Pooled and heterogeneity Chi^2 tests and t tests were performed on the sex ratio data using the Minitab statistical package ver. 1.1 (Minitab Inc., State College PA). Analysis of variance (ANOVA) was used to compare the overall sex ratios of the three species using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC) followed by Scheffe's Multiple Comparison test when $P<0.05$.

2.1.1.3 Results and Discussion

Patterns of semiochemical-mediated flight periods were found with all three species (Figs. 1 and 2). Two flight periods were found for *I. pini* at Shinnish Creek (Fig. 1A), consistent with published data of 2-3 generations per year (Reid 1955; Bright and Stark 1973; Livingston 1979; Furniss and Carolin 1980). At Spukune Creek only the second flight period was noted (Fig. 2A), presumably due to a lack of traps during the initial part of the season (before 31 May).

The first flight period of *I. pini*, occurring between 1 May and 10 June at Shinnish Creek, probably consisted of overwintered beetles emerging from the duff. The second flight period, occurring between 10 July and 17 Sept at both locations, appears to have been much larger than the first and encompassed the majority of *I. pini* flying through the year. Most of the initial part of this second flight probably consisted of newly-emerged, first-generation beetles. The generation time for *I. pini* ranges from 40 to 100 days in the field (Prebble 1933; Reid 1955; Thomas 1961; Schenk and Benjamin

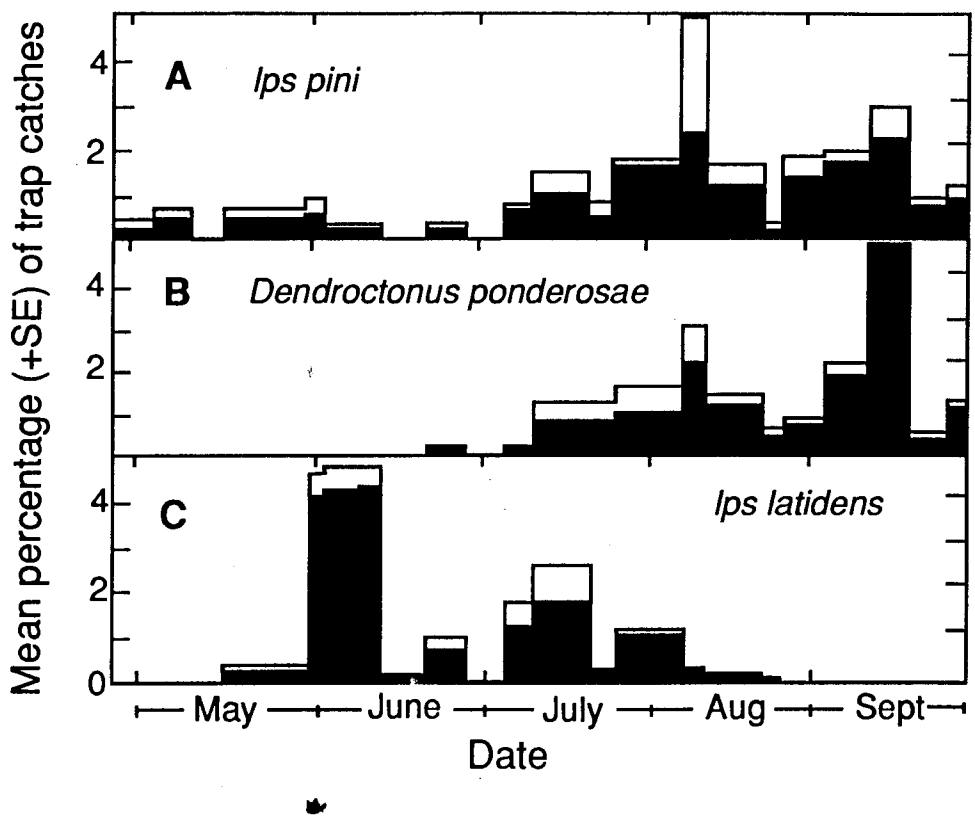


Figure 1. Semiochemical-mediated flight periods of three sympatric species of bark beetles in stands of lodgepole pine near Shinnish Creek BC during 1989. Daily means and SE are designated by solid and open bars, respectively.

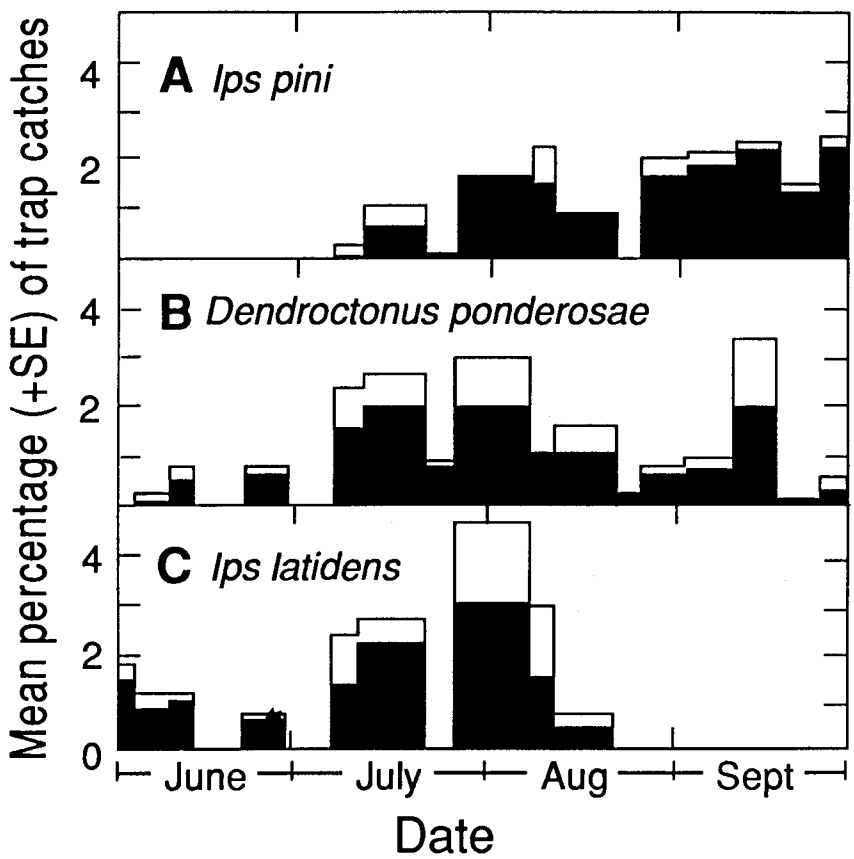


Figure 2. Semiochemical-mediated flight periods of three sympatric species of bark beetles in stands of lodgepole pine near Spukune Creek BC during 1989. Daily means and SE are designated by solid and open bars, respectively.

1969). Part of the middle part of this flight probably consisted of re-emerging, first-generation beetles. The later part of the flight should have contained primarily newly-emerged, second-generation beetles, searching for either brood material or feeding sites prior to entering overwintering sites (Bright and Stark 1973; Livingston 1979).

The flight period of *D. ponderosae* was similar at both sites (Figs. 1B and 2B), and consistent with a single generation per year (Safranyik et al. 1974; Furniss and Carolin 1980). The first activity began around 3 June, with the majority of flight between 1 July and 17 Sept.

In contrast to *I. pini* and *D. ponderosae*, the flight period of *I. latidens* was not the same at Shinnish and Spukune Creeks, with the major portion of the flight at Spukune Creek delayed by 30-50 days relative to that at Shinnish Creek. It is possible that the initial flight at Spukune Creek was missed due to lack of traps prior to 31 May. However, trap catches were close to nil in an experiment, testing the effect of monoterpenes on the attraction of *I. latidens* to ipsenol, located within 150 m of one of the replicates at Spukune Creek. Alternatively the delay was probably a consequence of climatic differences between the two locations. Sites at Spukune Creek tended to be cooler and wetter than those at Shinnish Creek, with east- or north-facing aspects. The sites at Shinnish Creek tended to face south. Cool temperatures at both sites tended to reduce trap catches to nil during the season, as seen during the periods of 13 to 21 June, 28 June to 5 July, 20 to 25 July, 10 to 24 Aug, and 15 to 22 Sept.

Differences in sex ratios were found among the three species (ANOVA, $F(2,12)$, $P<0.001$) (Table 1). The sex ratio of *D. ponderosae* caught over the entire monitoring period did not deviate significantly from 1:1 (t-test, df=4, $P=0.13$). In contrast the responding *I. pini* and *I. latidens* were predominantly female, particularly for *I. latidens* (Table 1).

The proportion of male *I. latidens* caught in traps did not change over the monitoring period (Fig. 3A) (pooled Chi^2 test, df=6, $P>0.05$). There was no

Table 1. Total proportion of males of *I. latidens*, *I. pini* and *D. ponderosae* caught in semiochemical-baited, multiple-funnel traps in stands of lodgepole pine near Princeton BC in 1989 (n=5).

Species	Mean (± SE) proportion of males [a]
Ips latidens	0.09 ± 0.023 a
Ips pini	0.29 ± 0.012 b
Dendroctonus ponderosae	0.42 ± 0.042 c

[a] Means followed by the same letter are not significantly different at P=0.05 (Scheffe's Multiple Comparison test).

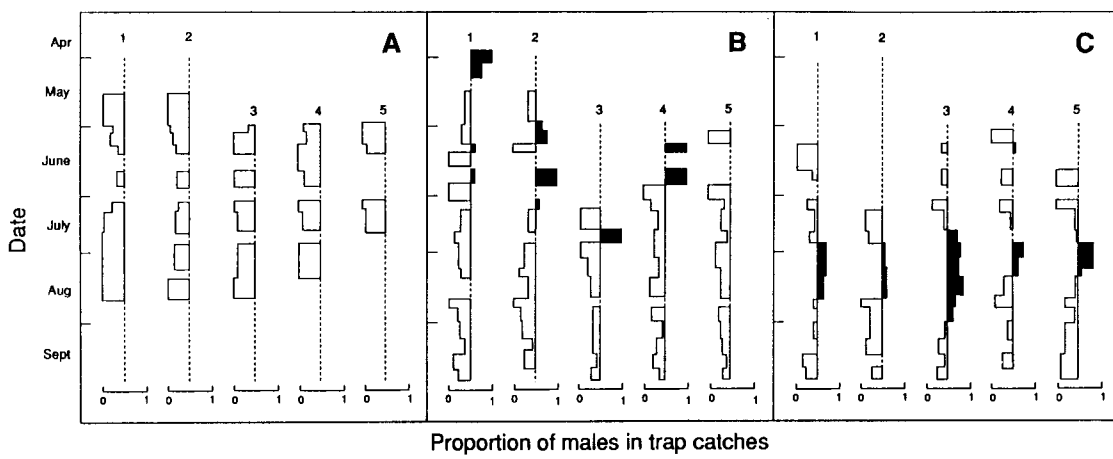


Figure 3. Proportion of males of *I. latidens* (A), *I. pini* (B) and *D. ponderosae* (C) caught in semiochemical-baited, multiple-funnel traps in stands of lodgepole pine near Princeton BC during 1989. Each vertical line represents a replicate with its identity printed at the top. Replicates 1, 2, and 5 were obtained near Shinnish Creek BC while replicates 3 and 4 were obtained near Spukune Creek BC.

heterogeneity among the replicates (heterogeneity Chi^2 test, df=11, P>0.10). However, there was significant heterogeneity in the association between sex ratio and date for both *I. pini* and *D. ponderosae* (heterogeneity Chi^2 test, df= 43 and 46, respectively, P<0.001 and P<0.001, respectively).

The sex ratio in catches of *I. pini* did not change for traps that only monitored the second flight period (replicates 3-5 in Fig. 3B) (pooled Chi^2 test, df=8, P>0.10). There was no heterogeneity amongst these three replicates (heterogeneity Chi^2 test, df=30, P>0.10). In contrast there was heterogeneity between replicates 1 and 2 (Fig. 3B) that monitored both flight periods (heterogeneity Chi^2 test, df=21, P<0.001). The sex ratio of *I. pini* in trap catches of both replicates changed over time (Chi^2 test, df=6 and 6, respectively, P<0.001 and P<0.01, respectively). The second flight period in all five replicates seemed consistently female-biased.

With *D. ponderosae* all five replicates (Fig. 3C) showed a significant association between time and sex ratio (Chi^2 test, df= 9, 4, 8, 7 and 5, respectively, P<0.005, P<0.005, P<0.001, P<0.025 and P<0.001, respectively). In all five replicates the season began with female-bias in the responding beetles, switched to male-bias between 19 July and 18 Aug, and then switched back to female-bias for the rest of the season. The overall sex ratio remained at 1:1 (Table 1).

In no case, however, does seasonal separation appear to be sufficient for achieving reproductive isolation (Figs. 1 and 2). The flight period of *D. ponderosae* did not overlap at all with the flight period of the overwintered population of *I. pini*, but it overlaped entirely with the flight of the first-generation *I. pini*. The flight period of *I. latidens* overlaped with part of the flights of both *I. pini* and *D. ponderosae*. Although only 29-67 % of *I. pini* responded to pheromones during the flight of *I. latidens*, 81-100 % of *I. latidens* responded during the flight of *I. pini*. The percentage of *I. latidens* responding to pheromones during the flight period of *D. ponderosae* was 90-93 % at Spukune Creek but only 28-58 % at Shinnish Creek. Similarly the percentage of

D. ponderosae responding to their pheromone during the flight period of *I. latidens* was 65-67 % at Spukune Creek but only 26-41 % at Shinnish Creek.

Seasonal separation is not sufficient to ensure species-specificity among these three species because of significant overlap in their respective flight periods. Since site variation is probably related to weather, and since weather patterns at any given site can vary from year to year, it is also quite probable that any separation that exists during one season may not occur in subsequent seasons, at least not to the same extent. Therefore some other species-specific factor is required to maintain reproductive isolation among individuals of these three species as well as minimise interspecific competition for resources.

2.2 SPECIES-SPECIFIC HOST KAIROMONES

Kairomones are interspecific semiochemicals (Brown et al. 1970) that convey contextual information (*sensu* Smith 1977). Natural selection has favored individuals that modify their behavior in response to kairomones when such a response results in a significant probability of increasing the individual's fitness relative to individuals that fail to modify their behavior (Brown et al. 1970; Whittaker and Feeny 1971; Nordlund and Lewis 1976; Shorey 1977; Nordlund 1981; Dicke and Sabelis 1988). The use of a semiochemical as a kairomone should not benefit the emitter; it should benefit only the responder. Natural selection has not favored individuals that use the compound to convey information to the receiver. It is not a form of communication since a message (or signal) has not been sent (W.J. Smith 1965; Blum 1974; Otte 1974; Smith 1977).

It is possible that the use of a kairomone undergoes continual evolution (Schoonhoven 1981), typical of other traits in predator-prey coevolution systems. An alternate, and more likely, explanation is that the production of a kairomone is in fact adaptive, a fairly-well entrenched view in ecology (Darwin 1859; Mayr 1974; Smith 1977; Krebs and Davies 1981), and that the apparent maladaptiveness, as seen in the context of a kairomone function, is offset by other benefits to the individual (Brown et al. 1970; Blum 1974; Pasteels 1982).

Such a relationship is apparent in the conflict between trees and bark beetles. Coniferous trees have various defenses against invasion by bark beetles (Berryman 1969; Shrimpton 1978; Cates and Alexander 1982; Payne 1983; Raffa and Berryman 1987). Intimately involved with these defenses is the fact that many compounds in the resin of conifers, such as monoterpenes, are toxic to beetles (R.H. Smith 1963, 1965; Reid and Gates 1970; Coyne and Lott 1976; Payne 1983; Raffa and Berryman 1983a; Raffa et al. 1985). In lesion areas immediately around bark beetle attacks, the more toxic monoterpenes, such as limonene, can increase in concentration (Shrimpton and Watson

1971; Shrimpton 1973; Wright et al. 1979; Raffa and Berryman 1982a,b,1983b; Hain et al. 1983) to levels that are detrimental to adults, brood and symbiotic fungi (Reid et al. 1967; Shrimpton and Whitney 1968; Berryman 1969; Wong and Berryman 1977; Shrimpton 1978). Such resistance mechanisms of conifers are beneficial in protecting trees from insect and fungal damage during most of their growing years (Shrimpton and Watson 1971). Hence any detriment or apparent maladaptiveness when monoterpenes are used as kairomones by bark beetles only occurs when the trees are overmature. In the case of lodgepole pine, a breakdown in defenses may in fact favor survivorship of lodgepole pine progeny (Raffa and Berryman 1987).

The relative proportions of monoterpenes in the phloem tissue of conifers varies dramatically between species (Mirov 1961). Monoterpenes are probably released when physical damage occurs to phloem tissue or possibly when the tissue is stressed. They are also released during mass attacks by bark beetles, either through the frass produced by successful beetles or from the resin flowing from severed resin canals. Bark beetles vary in the type of host material that they infest (Stark 1982). It is possible that significant variation in monoterpene composition might occur between various types of host material such as between stumps and small branches. Beetles should exploit such variation if it conveys information regarding the suitability of the resource for beetle invasion and brood production. Such information would aid in maintaining reproductive isolation. If all individuals of one species respond to the same specific blend of host kairomones, exclusive of individuals of other species, then the probability of mating with a conspecific should be high. Accordingly, we find that bark beetles do tend to show single-species mass aggregations on suitable host material.

Several species of bark beetles do use monoterpenes as kairomones (Borden 1982; Dickens et al. 1984; Byers et al. 1985, 1988; Schroeder and Eidmann 1987; Schroeder 1988; Volz 1988; Chénier and Philogène 1989; Schroeder and Lindelow 1989). Of three species common in stands of lodgepole pine, *Ips latidens*, *I. pini* and *Dendroctonus*

ponderosae, the least understood is *I. latidens*. Primary attraction of *I. latidens* to high-girdled lodgepole pine has been demonstrated but the compounds used to attract them were not identified (Miller et al. 1986).

Both *I. pini* and *D. ponderosae* have receptor cells on their antennae that are keyed to various monoterpenes. Two host monoterpenes, myrcene and α-pinene, activated cells in antennae of *I. pini* (Angst and Lanier 1979; Mustaparta et al. 1979). Antennae of *D. ponderosae* respond to α-pinene, β-pinene, camphene, 3-carene, myrcene and limonene (Whitehead 1986). Attraction to pheromones was increased by myrcene (Billings et al. 1976; Borden et al. 1987b) and 3-carene (Conn et al. 1983), and possibly by α-pinene, camphene and terpinolene (Pitman 1971; Billings et al. 1976).

2.2.1 Monoterpenes: Kairomones for *Ips latidens*

2.2.1.1 Objective and Hypotheses

My objective was to determine the effects of some monoterpenes commonly found in the phloem tissue of lodgepole pine, on the response of *I. latidens* to its own pheromone, ipsenol. I tested the three following hypotheses: 1) different monoterpenes would have different effects on the attraction of *I. latidens* to ipsenol; 2) β-phellandrene, the most common monoterpene in the phloem tissue of lodgepole pine (Shrimpton 1972, 1973), would be attractive alone or in combination with ipsenol; and 3) the effect of β-phellandrene would be tempered by a blend of five other monoterpenes (myrcene, 3-carene, α-pinene, β-pinene and terpinolene).

2.2.1.2 Materials and Methods

(+)-3-Carene, (-)-β-phellandrene, (±)-α-pinene, (-)-β-pinene and terpinolene (chemical purities, all >95%) were obtained from H.D. Pierce, Jr. (Dept. of Chemistry, Simon Fraser University). The chiralities of β-phellandrene and β-pinene are predominantly (-) in lodgepole pine (Mirov 1961). β-Myrcene (chemical purity, 98%) was obtained from Phero Tech Inc.(Delta BC). (±)-Ipsenol (chemical purity, 98%) was obtained from Bedoukian Research Inc. (Danbury CT).

Monoterpenes were released from closed, polyethylene, micro-centrifuge tubes (400 μL) (Evergreen Scientific, Los Angeles CA), each filled with a single monoterpene. The release rates for α-pinene, β-pinene, myrcene, 3-carene, β-phellandrene and terpinolene were approximately 8.9, 9.3, 22.3, 22.9, 29.3, and 29.5 mg/day, respectively, at 27 °C (determined by weight loss). An ipsenol release device consisted of a 10-cm length of C-flex® tubing (ID=1.6 mm; OD= 3.2 mm) (Concept Inc., Clearwater, FL), filled with a solution of ipsenol in ethanol, or ethanol (99%) for controls, and heat-pressure sealed at both ends. The release rate of ipsenol was approximately 0.6 mg/day at 24 °C (determined by collection of volatiles on Porapak-Q).

In all experiments, grids of 8-unit, multiple-funnel traps (Lindgren 1983) (Phero Tech Inc., Vancouver, B.C.) were set in mature stands of lodgepole pine near Princeton BC. Replicate grids were placed at least 100 m apart, and traps were spaced 10-15 m apart within each replicate grid. Each baited trap was suspended from a metal pole such that the top funnel of each trap was 1.3-1.5 m above ground. Sexes of captured *I. latidens* were determined by dissection and examination of genitalia.

The effects of various monoterpenes in combination with ipsenol were tested in Experiment 1. Ten replicates of nine traps per replicate, were set in grids of 3 x 3, from 24 May to 2 July, 1987. The treatments were as follows: 1) blank control; 2) ethanol control; 3) (±)-ipsenol; 4) ipsenol with 3-carene; 5) ipsenol with myrcene; 6) ipsenol

with β-phellandrene; 7) ipsenol with α-pinene; 8) ipsenol with β-pinene; and 9) ipsenol with terpinolene.

Experiment 2 compared the effect of β-phellandrene to that of the combination of the other five monoterpenes, with and without ipsenol. Ten replicates of eight traps each, were set in grids of 2 x 4, from 25 May to 2 July, 1987. The treatments were as follows: 1) ethanol control; 2) β-phellandrene alone; 3) combination of 3-carene, myrcene, α-pinene, β-pinene, and terpinolene; 4) all six monoterpenes; 5) (±)-ipsenol alone; 6) ipsenol with β-phellandrene; 7) ipsenol with 3-carene, myrcene, α-pinene, β-pinene and terpinolene; and 8) ipsenol with all six monoterpenes.

The data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). When necessary, trap catch data were transformed to remove heteroscedasticity. Catches of *I. latidens* were transformed by ln(Y+1) in Experiment 1 and $\sqrt[3]{Y}$ in Experiment 2. Sex ratio data were not transformed. Homoscedastic data were subjected to one-way analysis of variance (ANOVA) and Duncan's Multiple Range test when $P<0.05$. Orthogonal contrasts were performed on the trap catch data for *I. latidens* in Experiment 1. In Experiment 2, three-way, full-factorial ANOVA was performed to determine interaction effects.

2.2.1.3 Results

Monoterpenes had significant effects on the attraction of *I. latidens* to ipsenol (Fig. 4). The combination of β-phellandrene and ipsenol was the preferred treatment, increasing trap catches by >100% relative to ipsenol alone. Orthogonal contrasts discerned groupings in the data. The catches in traps baited with ipsenol and either 3-carene, myrcene, α-pinene or terpinolene seemed to be similar. As a group, their trap catches were compared to that of traps baited with ipsenol alone and the reduction was found to

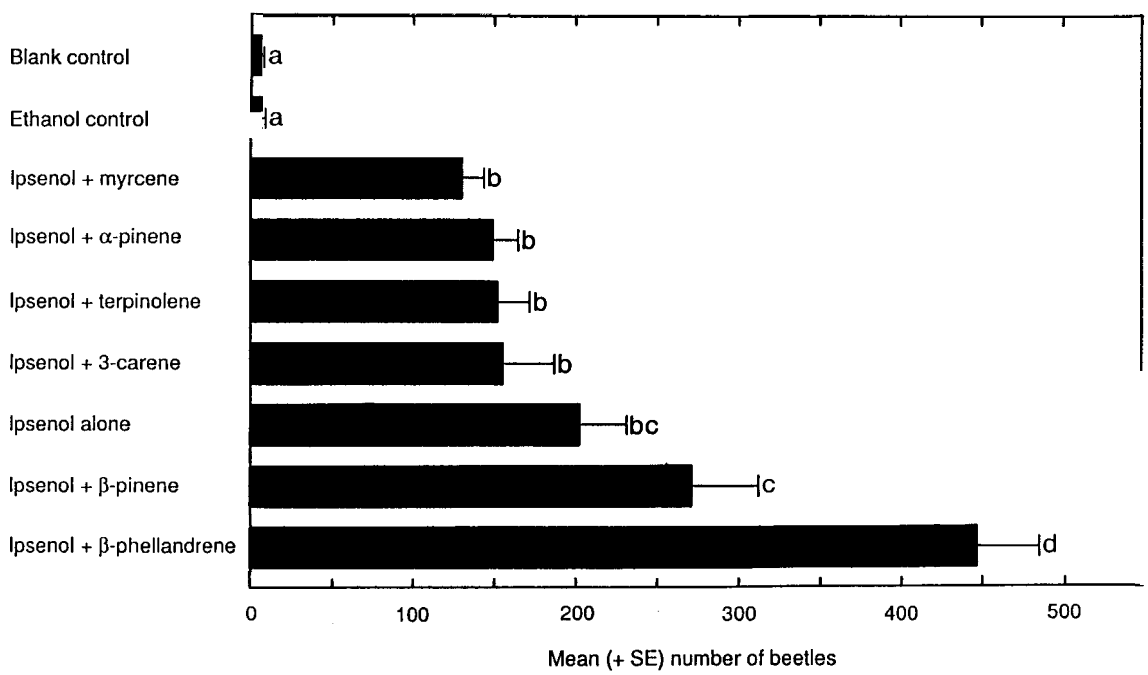


Figure 4. The effects of various monoterpenes on the attraction of *I. latidens* to ipsenol-baited, multiple-funnel traps near Princeton BC from 24 May to 2 July, 1987 (n=10). Means followed by the same letter are not significantly different at P=0.05 [Duncan's Multiple Range test on data transformed by ln(Y+1)].

be weakly significant (orthogonal contrast, ANOVA, P=0.074). There was no significant difference in the sex ratio of *I. latidens* amongst the different treatments (ANOVA, F(7,49), P=0.922). The mean (± SE) proportion of males in traps baited with ipsenol was 0.16 ± 0.010; significantly different from a 1:1 ratio (t test, P<0.001, df=69). *Ips latidens* was attracted to traps baited with β-phellandrene, even without ipsenol. β-Phellandrene with ipsenol was significantly more attractive than all other treatments (Fig. 5A). The interaction between ipsenol and β-phellandrene appears to be additive and not synergistic [ANOVA, F(1,71), P=0.937]. The relative increase in trap catches between the ethanol control and β-phellandrene is similar to the relative increase between ipsenol and ipsenol with β-phellandrene. Similarly, the relative increase in trap catches due to ipsenol was the same whether β-phellandrene was absent or present. The 5-terpene mix of 3-carene, myrcene, α-pinene, β-pinene and terpinolene, was not attractive and inhibited responses of *I. latidens* to ipsenol, β-phellandrene and the combination of ipsenol with β-phellandrene. Inhibition by the 5-terpene mix is consistent with the weak inhibition demonstrated by four of the five monoterpenes in Experiment 1 (Fig. 4).

The proportions of male *I. latidens* responding to traps baited with only monoterpenes were not significantly different from that to the ethanol control, although proportionally fewer males responded to the 5-terpene mix than to β-phellandrene (Fig. 5B). Whenever ipsenol was present, the sex ratio became strongly female biased, regardless of the monoterpene additives. There were weakly significant interactions between ipsenol and β-phellandrene, and between ipsenol and the 5-terpene mix [ANOVA, F(1,54), P=0.090 and P=0.038, respectively]. In both cases, the proportion of females tended to increase when ipsenol was presented with monoterpenes.

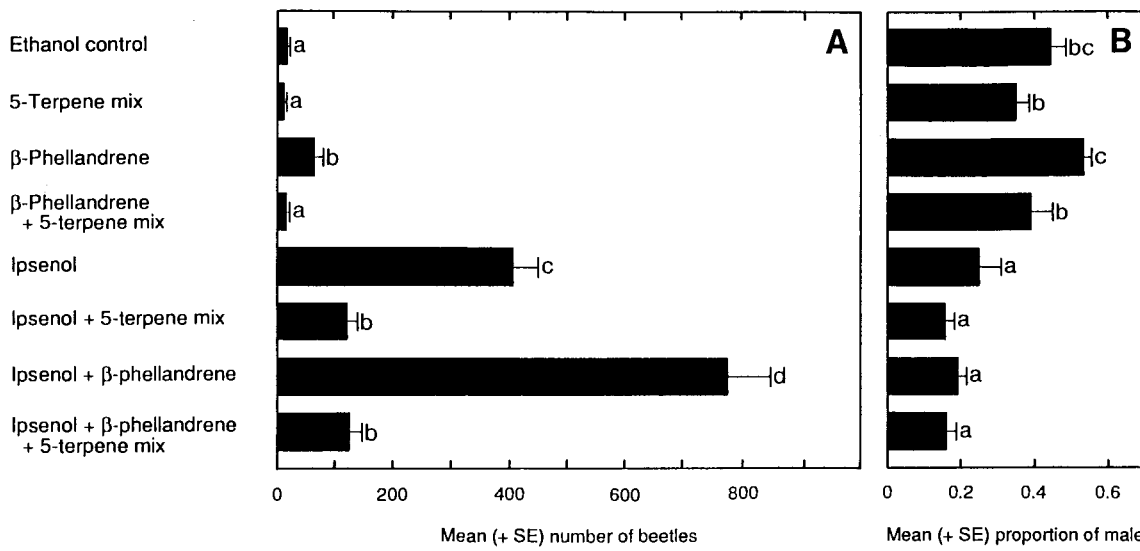


Figure 5. The interaction between β-phellandrene, a 5-terpene mix of 3-carene, α-pinene, β-pinene, terpinolene and myrcene, and (±)-ipsenol on the number (A) and sex ratio (B) of *I. latidens* captured in multiple-funnel traps near Princeton BC from 25 May to 2 July, 1987 (n=10). Catch numbers were transformed by $\sqrt[3]{Y}$ for analyses. Means followed by the same letter are not significantly different at P=0.05 (Duncan's Multiple Range test).

2.2.1.4 Discussion

My results support the hypothesis that host kairomones are used by *I. latidens* (Fig. 4). β-Phellandrene was attractive alone and increased attraction to sources of ipsenol (Fig. 5A). In contrast, the four other monoterpenes negated the effects of β-phellandrene (Fig. 5A). The combination of all six monoterpenes with ipsenol significantly reduced trap catches relative to traps baited with ipsenol alone. These results are the clearest demonstration that monoterpenes are used as kairomones by a species of *Ips* since monoterpenes were implicated as attractants for *I. typographus* (Rudinsky et al. 1971a,b; Tomescu et al. 1979) and *I. grandicollis* (Werner 1972a,c). Conclusive support of this hypothesis requires the determination of the volatiles actually released from host material suitable for *I. latidens.*

Ips latidens seems to prefer drier phloem than that used by *I. pini* (Miller and Borden 1985). β-Phellandrene is the major monoterpene in phloem of lodgepole pine (Shrimpton 1972,1973). As phloem dries, therefore, β-phellandrene would most likely remain above the threshold level required for perception at some distance from a potential host. In fresh hosts, other monoterpenes may be present at levels above thresholds and may indicate non-preferred phloem conditions.

Ips latidens does not show a sex-specific response to sources of monoterpenes (Fig. 5B). The sex ratio of *I. latidens* emerging from infested lodgepole pine is approximately 1:1 (Miller and Borden 1985). The sex ratio of *I. latidens* caught in control traps was not significantly different from 1:1 (t test, P=0.26, df=3). Similarly, *D. pseudotsugae* (Furniss and Schmitz 1971) and *Tomicus piniperda* (Byers et al. 1985) also do not show sexual specificity in their responses to monoterpenes. Kairomones convey contextual information about the environment and therefore should not necessarily be expected to be sex specific.

In contrast, *I. latidens* did exhibit a strong female bias in responding to ipsenol-baited traps (Fig. 5B). This sex-specificity in responses to ipsenol may reflect differential benefits to females and males, as in the six-spined spruce bark beetle, *Pityogenes chalcographus*, in Europe (Byers et al. 1988).

In polygynous species of bark beetles, such as *I. latidens* and *P. chalcographus,* the production of pheromone by males, particularly in the presence of monoterpenes, signifies to females that there are galleries available for breeding. As long as pheromone is produced, females should have galleries to enter, particularly since several females can join the same male (Kirkendall 1983). Males, on the other hand, are looking for access to suitable breeding material in order to gain access to females. Sources of pheromone are attractive since males can usually establish galleries in adjacent phloem on the same log or tree. However, the number of available sites is limited and the relative benefits to males should decrease as the available sites are taken; benefits to females should not be expected to decrease. The production of pheromone should increase as more males establish galleries. Increases in the attraction of males may still occur but not to the same extent as increases in the attraction of females, as occurs in *I. paraconfusus* and *P. chalcographus* (Byers 1983; Byers et al. 1988). Schlyter et al. (1987b) found that significantly more female than male *I. typographus* landed on pheromone-baited traps, even though both sexes showed equal long-distance attraction to the same pheromone sources.

When all sites are occupied by males, then males will no longer benefit by responding to pheromone sources. However, females will still benefit as the continued production of pheromones probably signifies that some males are still looking for females. Pheromone production by males of four polygynous species, *I. paraconfusus, I. grandicollis, I. calligraphus* and *I. typographus*, seems to decrease as males acquire females, and apparently ceases as harems are filled (Borden 1967; Werner 1972b; Svihra 1982; Birgerrson et al. 1984; Birgerrson and Leufven 1988; Byers 1989b).

2.2.2 Monoterpenes: Kairomones for *Ips pini*

2.2.2.1 Objective and Hypotheses

My objective was to determine the effects of some monoterpenes, commonly found in the phloem tissue of lodgepole pine (Shrimpton 1972), on the behavioral responses of *Ips pini*. I tested the four following hypotheses: 1) different monoterpenes would have different effects on the attraction of *I. pini* to its pheromone, ipsdienol; 2) β-phellandrene, the most common monoterpene in the phloem tissue of lodgepole pine (Shrimpton 1972, 1973), would be attractive alone or in combination with ipsdienol; 3) attraction to ipsdienol would not be synergised by β-phellandrene; and 4) one, several or all of five other monoterpenes (myrcene, α-pinene, β-pinene, terpinolene and γ-terpinene) would be attractive alone and/or in combination with ipsdienol.

2.2.2.2 Materials and Methods

In 1987 and 1988, (+)-3-carene, (+)-limonene, (-)-β-phellandrene, racemic α-pinene, (-)-β-pinene and terpinolene (chemical purities, all >95%) were obtained from H.D. Pierce, Jr. (Dept. of Chemistry, Simon Fraser University). β-Myrcene and racemic ipsdienol (chemical purities, both 98%) were obtained from Phero Tech Inc. (Delta BC). In 1989 (+)-3-carene, (-)-α-pinene, (-)-β-pinene and γ-terpinene (chemical purities, all >95%) were obtained from Aldrich Chemical Co. (Milwaukee WI), while terpinolene (chemical purity, 98%) was obtained from D. Vanderwel (Dept. of Chemistry, Simon Fraser University). Ipsdienol bubble-cap lures, containing a solution of racemic ipsdienol (chemical purity, 98%) in 3-butanediol, were obtained from Phero Tech Inc. (Delta BC). The chiralities of β-phellandrene and β-pinene are predominantly (-) in lodgepole pine (Mirov 1961).

In Experiment 1, monoterpenes were released from closed, polyethylene micro-centrifuge tubes (400 μL) (Evergreen Scientific, Los Angeles CA), each filled with a single monoterpene. The release rates for α-pinene, β-pinene, myrcene, 3-carene, limonene, β-phellandrene and terpinolene were approximately 8.9, 9.3, 22.3, 22.9, 25.5, 29.3 and 29.5 mg/day, respectively, at 27-30 °C (determined by weight loss). In Experiment 2, β-phellandrene was released in two fashions to obtain two different release rates: 1) two closed, polyethylene micro-centrifuge tubes (each 400 μL) per trap; and 2) one closed, polyethylene screw-cap bottle (15 mL) (Ampak Inc., Richmond BC) per trap. The release rates were approximately 59 and 450 mg per day, respectively, at 27-30 °C. In Experiment 3, β-phellandrene was released in three fashions: 1) one open, polypropylene, micro-centrifuge tube (1.5 mL) (Quality Scientific Plastics, Petaluma CA) per trap, containing five 2-cm-long glass capillaries (ID=1.5 mm; OD=1.8 mm), each sealed at one end and filled with β-phellandrene; 2) five closed, polyethylene, micro-centrifuge tubes (1.8 mL) (Evergreen Scientific, Los Angeles CA) per trap; and 3) one closed, polyethylene, screw-cap bottle (15 mL) per trap. The release rates were approximately 3, 40 and 450 mg/day, respectively, at 27-30 °C. In Experiments 4-8, polyethylene transfer pipettes (3.5 mL) (Saint-Amand Mfg. Co., San Fernando CA) were filled with monoterpenes and heat-pressure sealed. The release rates of β-pinene, myrcene, α-pinene, 3-carene, β-phellandrene, γ-terpinene and terpinolene were approximately 121, 135, 143, 182, 187, 293 and 343 mg/day, respectively, at 27-30 °C (determined by weight loss).

In Experiments 1-3, ipsdienol was released from 10-cm-lengths of C-flex ® tubing (ID=1.6 mm; OD=3.2 mm) (Concept Inc., Clearwater FL), filled with a solution of ipsdienol in ethanol (chemical purity, 99%), and heat-pressure sealed at both ends. In Experiments 1-2, the release rate of ipsdienol was approximately 0.6 mg/day at 24 °C (determined by collection of volatiles on Porapak-Q). The release rates for the devices used in Experiment 3 (approximately 6, 60, and 600 μg/day at 24 °C) were obtained by

varying the concentration of ipsdienol in ethanol. In Experiments 4-8, the release rate of ipsdienol from bubble-cap lures was approximately 0.2-0.3 mg/day at 24 °C (determined by collection of volatiles on Porapak-Q).

In all experiments, grids of multiple-funnel traps (Lindgren 1983) were set in mature stands of lodgepole pine. Replicate grids were placed at least 100 m apart, and traps were spaced 10-15 m apart within each replicate. Each trap was suspended between trees by rope such that the top funnel of each trap was 1.3-1.5 m above ground. No trap was within 2 m of any tree. Sexes of captured *I. pini* were determined using declivital characters (Lanier and Cameron 1969).

Various monoterpenes were tested in Experiment 1 in order to distinguish their relative effects. Five replicates of eight 8-unit traps per replicate, were set near Princeton BC, in grids of 2 x 4, from 29 May to 2 July, 1987. The treatments were as follows: 1) ipsdienol alone; 2) ipsdienol with α-pinene; 3) ipsdienol with β-pinene; 4) ipsdienol with 3-carene; 5) ipsdienol with myrcene; 6) ipsdienol with terpinolene; 7) ipsdienol with β-phellandrene; and 8) ipsdienol with limonene.

Experiment 2 tested for effects of β-phellandrene as a kairomone in primary and secondary attraction of *I. pini*, as well as for interactions between ipsdienol, β-phellandrene and locality. Three replicates of six 12-unit traps per replicate were set in grids of 2 x 3 at each of three localities in British Columbia in 1988: 1) near Princeton from 24 Aug to 4 Sept; 2) near Jaffray from 25 Aug to 27 Sept; and 3) near Williams Lake from 27 to 31 Aug. The treatments were as follows: 1) blank control; 2) β-phellandrene (59 mg/day); 3) β-phellandrene (450 mg/day); 4) ipsdienol alone; 5) ipsdienol with β-phellandrene (59 mg/day); and 6) ipsdienol with β-phellandrene (450 mg/day).

Experiment 3 tested for response of *I. pini* to different doses of ipsdienol and β-phellandrene, as well as for interaction between ipsdienol, β-phellandrene and locality. Two replicates of nine 12-unit traps per replicate were set in grids of 3 x 3 at each of

three localities in British Columbia in 1988: 1) near Princeton from 19 Aug to 1 Sept; 2) near Jaffray from 25 Aug to 27 Sept; and 3) near Williams Lake from 27 to 31 Aug. The treatments were the nine binary combinations of ipsdienol (6, 60, and 600 μg/day) with β-phellandrene (3,40, and 450 mg/day), with each of the release rates for a given chemical occurring only once in any row or column of each grid.

Experiments 4-8 tested for the effects of γ-terpinene, 3-carene, terpinolene, α-pinene and β-pinene, respectively, on the primary and secondary attraction of *I. pini*. In each experiment replicates of four 12-unit funnel traps per replicate were set in grids of 2 x 2 near Princeton BC during 1988. Experiment 4 tested γ-terpinene with eight replicates set from 20 July - 6 Aug to 10 Aug - 2 Sept. Experiments 5-8 tested 3-carene, terpinolene, α-pinene and β-pinene, respectively, with ten replicates per experiment set from 10 Aug - 3 Sept to 3-26 Sept. The treatments were as follows: 1) blank control; 2) monoterpene alone; 3) ipsdienol alone; and 4) ipsdienol with monoterpene.

The data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). When necessary, trap catch data were transformed to remove heteroscedasticity. Trap catches of *I. pini* were transformed by $\sqrt[3]{Y}$ in Experiment 1, $\ln(Y+1)$ in Experiments 2-6, and $-1/\sqrt{Y}$ in Experiments 7-8. Catches of *D. ponderosae* were transformed by $-1/\sqrt{Y}$. Sex ratio data for *I. pini* were transformed by $\arcsin\sqrt{Y}$ in Experiments 1-3. Homoscedastic data from Experiments 1-3 were subjected to either one-way, two-way or three-way full-factorial analysis of variance (ANOVA). Data from Experiments 4-8 were analysed by ANOVA using the following effects: block, ipsdienol treatment, monoterpene treatment and the interaction between ipsdienol and monoterpene treatments. Sex ratios in these later five experiments were analysed using only block and monoterpene treatments on catch data for ipsdienol-baited traps. Three orthogonal contrasts were performed for Experiment 1 while Duncan's Multiple Range tests were used in Experiments 2-3 when $P<0.05$.

2.2.2.3 Results

β-Phellandrene had a significant effect on trap catches of *I. pini* in Experiments 1-3. In Experiment 1, catches to traps baited with ipsdienol and β-phellandrene were significantly higher than catches to traps baited with ipsdienol and any other monoterpene (Fig. 6). There were no significant differences between the ipsdienol treatment and either the ipsdienol+β-phellandrene treatment [orthogonal contrast, ANOVA, P=0.226] or the remaining treatments [orthogonal contrast, ANOVA, P=0.488].

Jaffray data were omitted from analyses in Experiments 2-3 because only 12 *I. pini* were caught. In Experiment 2, β-phellandrene alone significantly increased trap catches (Table 2; Figs. 7A,C). In Princeton, traps with baits releasing β-phellandrene at a high rate were preferred over those with a low release rate, while catches in blank control traps were intermediate (Fig. 7A). In Williams Lake, traps with baits releasing β-phellandrene at either rate were preferred over blank controls (Fig. 7C). There was an additive, not synergistic, interaction between ipsdienol and β-phellandrene on the response of *I. pini* (Table 2). Significant interactions occurred between location and β-phellandrene treatments, and between location, β-phellandrene and ipsdienol treatments.

In Experiment 3, responses of *I. pini* increased as the release rates of ipsdienol and β-phellandrene increased (Table 3; Fig. 8A,B). There were significant differences between Princeton and Williams Lake in the magnitude of the increase (Table 3). However, in both cases the preferred treatments were those with high release rates of ipsdienol and β-phellandrene (Duncan's Multiple Range test, P=0.05). The interaction between ipsdienol and β-phellandrene was not synergistic (Table 3).

Three of the monoterpenes tested in Experiments 4-8 had significant effects on the attraction of *I. pini* (Fig. 9). 3-Carene significantly increased attraction of *I. pini*,

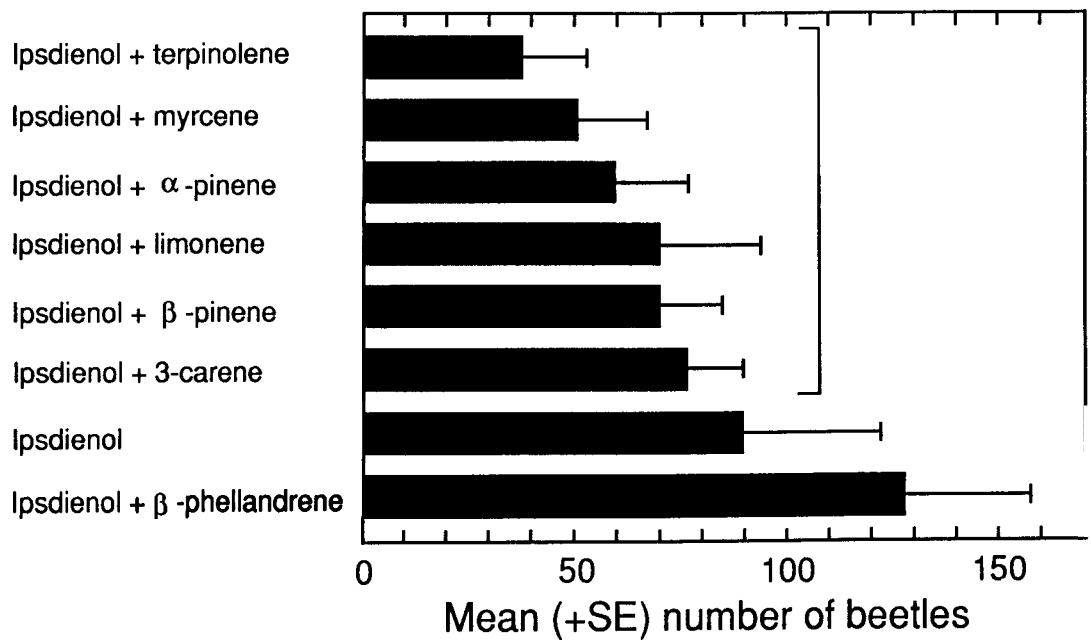


Figure 6. The effect of various monoterpenes on the attraction of *I. pini* to ipsdienol-baited, multiple-funnel traps in Experiment 1 near Princeton BC from 24 May to 2 July, 1987. Means grouped by a line are significantly different from the treatment of ipsdienol+β-phellandrene at P=0.027 [orthogonal contrast, ANOVA, F(1,32), on data transformed by $\sqrt[3]{Y}$ (n=5)].

Table 2. Analysis of variance (ANOVA) on the effects of location (Princeton and Williams Lake BC), ipsdienol (0.6 mg/day) and β-phellandrene (59 and 450 mg/day) on the number and sex ratio of *I. pini* captured in multiple-funnel traps in Experiment 2 in 1988.

	Trap catch [a]			Proportion of males [b]		
Source	df	F	P	df	F	P
Location (A)	1	32.24	<0.001	1	0.49	0.493
Ipsdienol (B)	1	281.46	<0.001	1	12.17	0.003
β-Phellandrene (C)	2	5.66	0.010	2	0.45	0.647
A * B	1	1.79	0.193	1	0.02	0.896
A * C	2	5.07	0.015	2	0.38	0.692
B * C	2	1.71	0.203	2	2.68	0.094
A * B * C	2	2.94	0.072	0		
Error	24			19		

[a] Data transformed by $\ln(Y+1)$.

[b] Data transformed by $\arcsin\sqrt{Y}$.

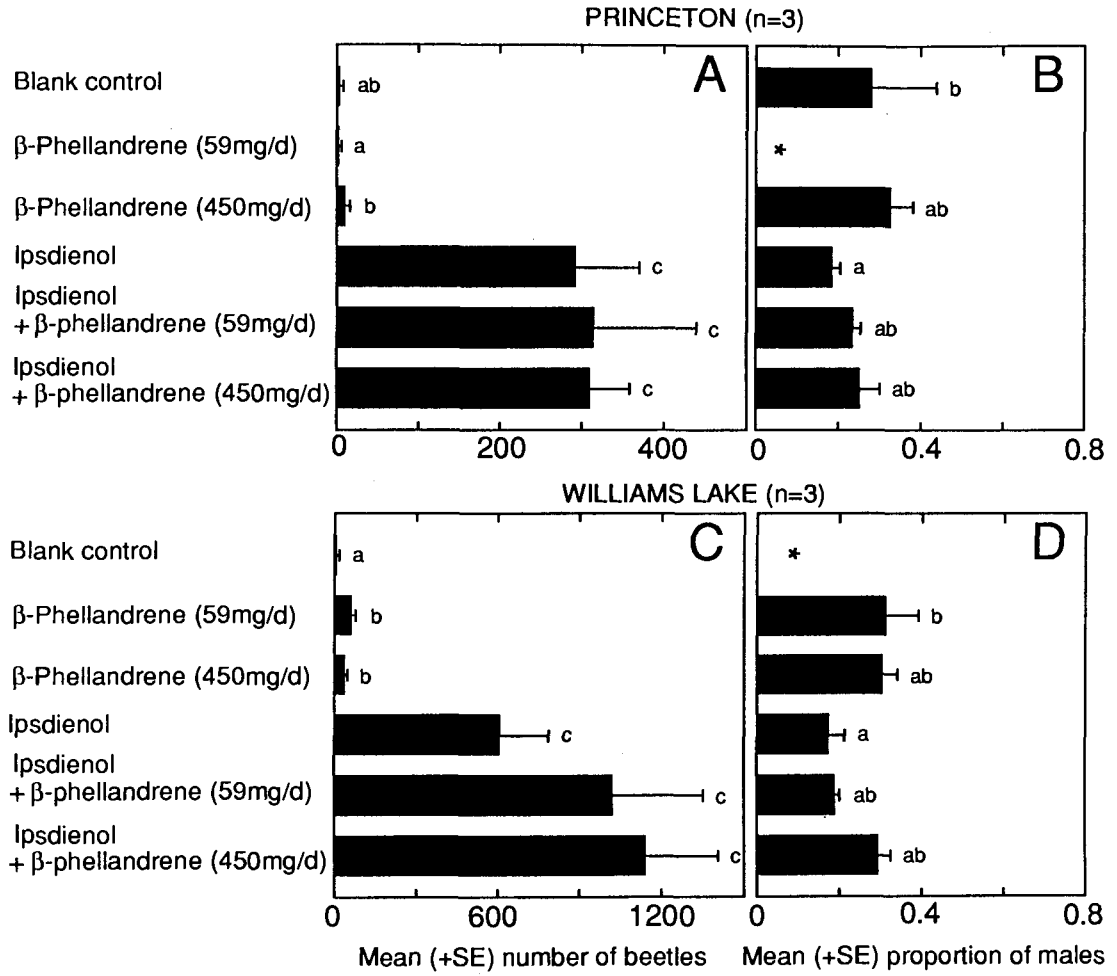


Figure 7. The effect of β-phellandrene, with or without ipsdienol, on the number (A,C) and sex ratio (B,D) of *I. pini* captured in multiple-funnel traps in Experiment 2 near Princeton (A,B) and Williams Lake BC (C,D) from 24 Aug to 4 Sept and 27 to 31 Aug, 1988, respectively. Mean trap catches from the same location followed by the same letter are not significantly different at P=0.05 [Duncan's Multiple Range test on data transformed by ln(Y+1)]. Mean proportions of males, in traps at the same location, followed by the same letter are not significantly different at P=0.05 (Duncan's Multiple Range test on data transformed by arcsin$\sqrt{Y}$). The proportions of males for treatments with low trap catches (*)were not included in the analyses.

Table 3. Analysis of variance (ANOVA) on the effects of location (Princeton and Williams Lake BC), release rate of ipsdienol (6, 60, and 600 μg/day) and release rate of β-phellandrene (3, 40, and 450 mg/day) on the number and sex ratio of *I. pini* captured in multiple-funnel traps in Experiment 3 in 1988.

	Trap catch [a]			Proportion of males [b]		
Source	df	F	P	df	F	P
Location (A)	1	15.45	0.001	1	5.18	0.035
Ipsdienol (B)	2	48.08	<0.001	2	17.79	<0.001
β-Phellandrene (C)	2	17.57	<0.001	2	0.64	0.538
A * B	2	4.93	0.020	2	0.16	0.857
A * C	2	0.83	0.454	2	0.46	0.641
B * C	4	0.73	0.582	4	1.32	0.299
A * B * C	4	0.56	0.698	4	0.96	0.456
Error	18			18		

[a] Data transformed by $\ln(Y+1)$.

[b] Data transformed by $\arcsin\sqrt{Y}$.

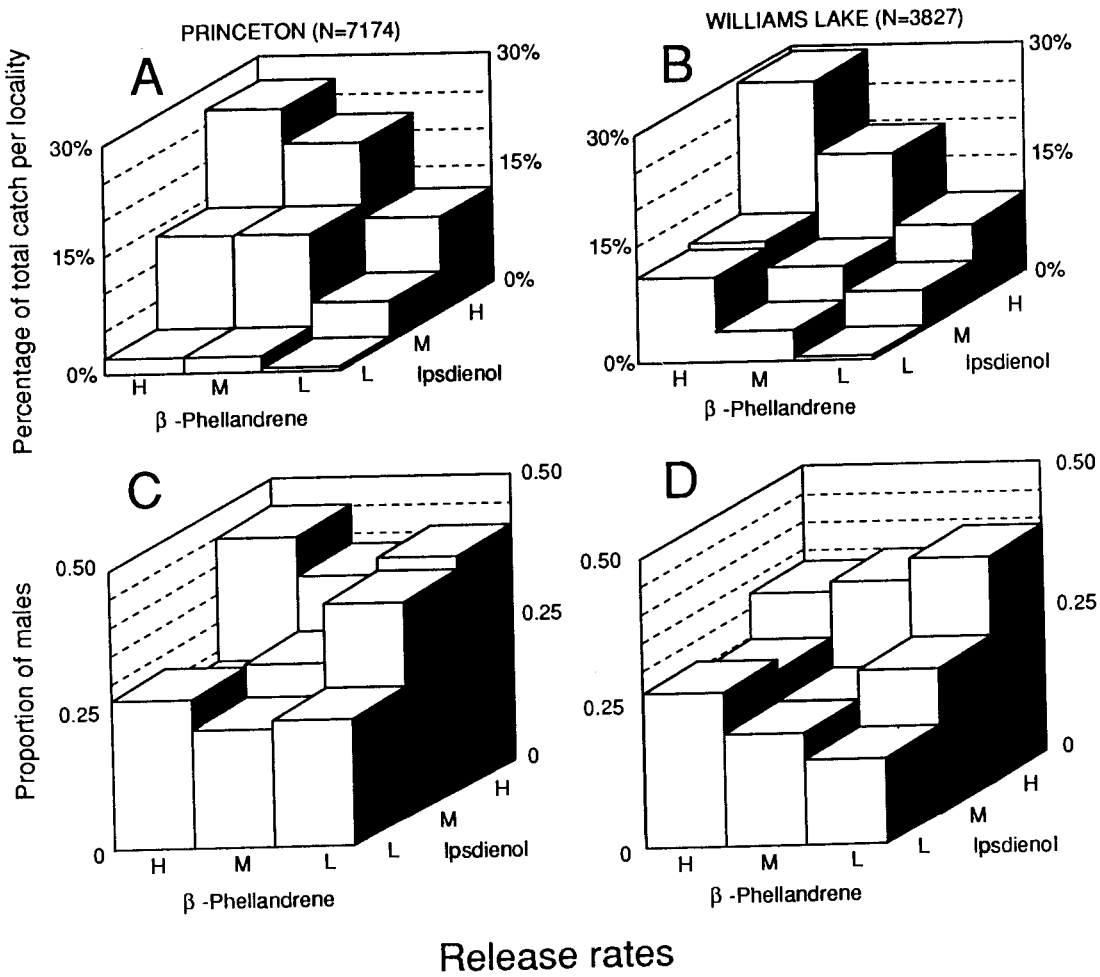


Figure 8. The interaction between β-phellandrene and ipsdienol on the number (A,B) and sex ratio (C,D) of *I. pini* captured in multiple-funnel traps in Experiment 3 near Princeton (A,C) and Williams Lake BC (B,D) from 19 Aug to 1 Sept, 1988, and 27-31 Aug, 1988, respectively. Release rates were 3 (L), 40 (M), and 450 (H) mg/day for β-phellandrene and 6 (L), 60 (M), and 600 (H) μg/day for ipsdienol.

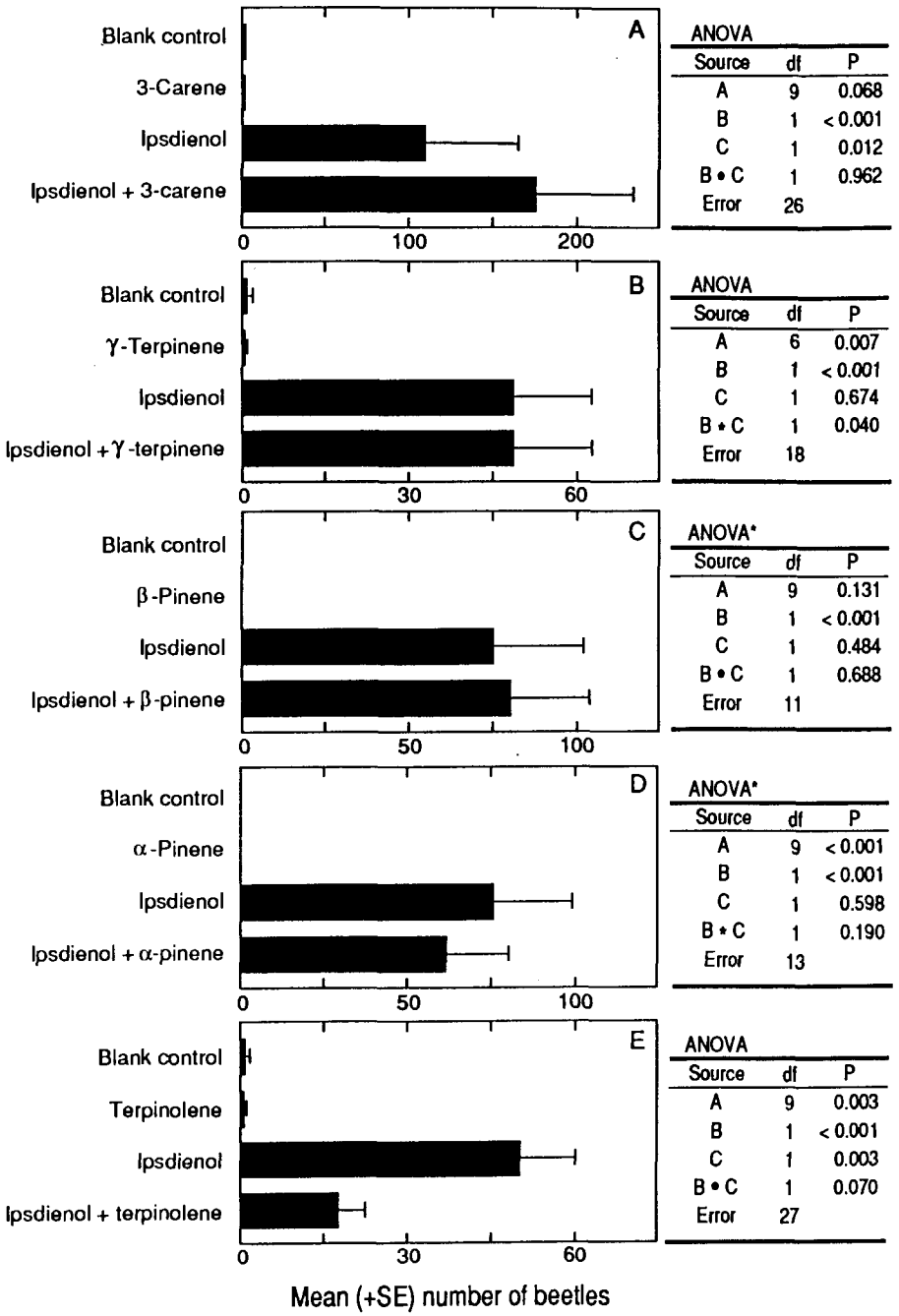


Figure 9. The effects of 3-carene (A), γ-terpinene (B), β-pinene (C), α-pinene (D) and terpinolene (E), with or without ipsdienol, on the attraction of *I. pini* to multiple-funnel traps in Experiments 4-8, respectively, near Princeton BC in 1989. Data were transformed by either ln(Y+1) (A,B, and E) or $-1/\sqrt{Y}$ (C and D), and subjected to ANOVA using the following sources of variance: block (A), ipsdienol treatment (B), monoterpene treatment (C), and the interaction between ipsdienol and monoterpene treatments (B*C).

with and without ipsdienol (Fig. 9A); there was no interaction between 3-carene and ipsdienol. In contrast there was a significant interaction between γ-terpinene and ipsdienol (Fig. 9B). However it is not clear whether this interaction is a consequence of a reduction in trap catch relative to the blank control or an increase relative to traps baited with only ipsdienol. There was no effect of α- and β-pinene on the attraction of *I. pini* (Figs. 9C,D). However terpinolene significantly reduced trap catches, with and without ipsdienol (Fig. 9E).

The effect of β-phellandrene on the sex ratio of responding *I. pini* was variable. In Experiment 1, the proportion of males in trap catches (mean ± SE = 0.38 ± 0.013) was not affected by the presence of monoterpenes [ANOVA, F(7,32), P=0.582]. In Experiments 4-8 there was no significant effect of monoterpenes on the sex ratio of *I. pini* caught in ipsdienol-baited traps [ANOVA, F(1,9), F(1,6), F(1,7), F(1,9), and F(1,9), respectively, P=0.808, P=0.765, P=0.118, P=0.943, and P=0.453, respectively]. In Experiment 2, the presence of ipsdienol in traps significantly reduced the proportion of males (Table 2; Figs. 7B,D). β-Phellandrene did not affect the sex ratio of beetles caught, although the interaction between ipsdienol and β-phellandrene was weakly significant. In contrast, the proportion of males caught in traps in Experiment 3 increased with an increase in release rate of ipsdienol but showed no effect due to the release rate of β-phellandrene (Table 3; Figs. 8C,D).

2.2.2.4 Discussion

My results support the hypothesis that β-phellandrene is used as an attractive host kairomone by *I. pini*. Traps with baits releasing β-phellandrene at a high rate were more attractive to *I. pini* than blank control traps (Figs. 7A,B). β-Phellandrene significantly increased catches of *I. pini* to traps baited with ipsdienol (Table 2; Figs. 7C and 3A,B) except for one location on one occasion when it failed to substantiate these results (Fig. 7A). In addition my results suggest that 3-carene and terpinolene may also be used as attractive and repellent kairomones, respectively. Attraction of *I. pini* was significantly increased by 3-carene (Fig. 9B) but significantly decreased by terpinolene (Fig. 9E). High levels of β-phellandrene and 3-carene may indicate suitable host material while high levels of terpinolene may indicate unsuitable material. Conclusive support of these hypotheses requires the determination of the volatiles actually released from host material suitable for *I. pini*.

The effect of the interactions between ipsdienol and β-phellandrene, and between ipsdienol and 3-carene, on the response of *I. pini* were additive. Similarly, *I. latidens* showed an additive effect to the combination of its pheromone, ipsenol, and β-phellandrene (Chap. 2.3.1). There is no evidence of either synergy or saturation in responses by either species. In both cases, the proportional increase in response due to the presence of a kairomone was the same with the respective pheromone as without it.

2.2.3 Dose-dependent and species-specific responses of *Ips latidens*, *I. pini* and *Dendroctonus ponderosae* to monoterpenes

2.2.3.1 Introduction

The question of the use of monoterpenes as kairomones by bark beetles has received considerable attention but few conclusive answers (D.L. Wood 1982). Problems with experimentation on monoterpenes have included poor design and low power. Various experiments with *Dendroctonus ponderosae* have neglected tests of significance (Pitman 1971) or the use of appropriate controls (Billings et al. 1976; Conn et al. 1983). Some of my experiments with *Ips latidens* and *I. pini* were confounded by the potential interactions between monoterpenes (Experiment 1, Chap. 2.2.1, and Experiment 1, Chap. 2.2.2), thereby minimising the possibility of detecting weak attraction or repulsion. In Experiment 2, Chap. 2.2.1, and Experiments 2 and 4-8, Chap. 2.2.2, I attempted to improve the power of my tests by segregating monoterpenes into separate experiments. However, a lack of response may have been a consequence of an inappropriate release rate rather than a lack of activity. Testing the effect of compounds over a broad, realistic range of release rates should be a fundamental concern in understanding the chemical ecology of organisms. Additionally, if monoterpenes are tested separately over a broad range of release rates then two powerful statistical procedures (regression analyses and orthogonal contrasts) can be used to discern effects. In Experiment 3, Chap. 2.2.2, *I. pini* exhibited dose-dependent responses to both β-phellandrene and ipsdienol, a type of response suited for regression analysis.

Geographic variation has also confounded generalisations regarding the use of kairomones. In stands of western white pine, α-pinene, camphene and myrcene seemed to increase the attraction of *D. ponderosae* to traps baited with the pheromone, *trans*-verbenol, while β-pinene, 3-carene and limonene appeared to be inactive (Pitman 1971). However, in stands of ponderosa pine, 3-carene seemed to increase attraction

while α-pinene had no apparent effect (Billings et al. 1976). Similarly in stands of lodgepole pine, α-pinene had no effect while 3-carene significantly increased catches of *D. ponderosae* in traps baited with *trans*-verbenol (Conn et al. 1983).

My objective was to assay the effects of various monoterpenes on the attraction of *I. latidens*, *I. pini* and *D. ponderosae* in stands of lodgepole pine. *Dendroctonus ponderosae* is attracted to 3-carene (Conn et al. 1983) and myrcene (Borden et al. 1987b). β-Phellandrene is attractive to both *I. latidens* and *I. pini* (Chaps. 2.2.1 and 2.2.2). Therefore, I tested 3-carene, myrcene and β-phellandrene on all three species to confirm their relative activities. In addition I tested four other monoterpenes (α-pinene, β-pinene, γ-terpinene and terpinolene) commonly found in the phloem tissue of lodgepole pine (Shrimpton 1972, 1973). My design tested only one monoterpene per experiment over a broad range of release rates (3 to 4 orders in magnitude) and in the presence of pheromones. I tested the two following hypotheses: 1) all three species would show dose-dependent responses (responses that are directly or inversely proportional to release rates of monoterpenes); and 2) evidence of species-specificity would be obvious in the combinations of attractive and repellent kairomones.

2.2.3.2 Materials and Methods

β-Myrcene (chemical purity, 98%) was obtained from Phero Tech Inc. (Delta BC). In 1988, (+)-3-carene, (-)-β-phellandrene, racemic α-pinene, (-)-β-pinene, γ-terpinene and terpinolene (chemical purities, all >95%) were obtained from H.D. Pierce, Jr. (Dept. of Chemistry, Simon Fraser University). In 1989, (+)-3-carene, (-)-α-pinene, (-)-β-pinene and γ-terpinene (chemical purities, all >95%) were obtained from Aldrich Chemical Co. (Milwaukee WI) while terpinolene (chemical purity, 94%) was obtained from D. Vanderwel (Dept. of Chemistry, Simon Fraser University). Phero Tech Inc. (Delta BC) supplied the following: 1-2) (±)-ipsenol and (±)-ipsdienol (chemical

purities, both 98%); 3) ipsenol, bubble-cap lures containing a solution of (±)-ipsenol (chemical purity, 98%) in 3-butanediol; 4) polyethylene, bubble-cap lures containing a 13:87 mix of *cis*- and *trans*-verbenol [chemical purities, both 98%; chiral ratios, both 83:17 (-):(+)]; and 5-6) two types of *exo*-brevicomin lures (chemical purity, 98%).

The following devices were used to release monoterpenes: 1-2) open, polypropylene, micro-centrifuge tubes (1.5 mL) (Quality Scientific Plastics, Petaluma CA) containing either one or five 2-cm-long, glass, capillary tubes (ID=1.5 mm; OD=1.8 mm), each sealed at one end and filled with monoterpene; 3-5) closed, polyethylene, micro-centrifuge tubes (either 0.25, 0.4 or 1.8 mL) (Evergreen Scientific, Los Angeles CA), each filled with monoterpene; 6) polyethylene, transfer pipettes (3.5 mL) (Saint-Amand Mfg. Co., San Fernando CA), each filled with monoterpene and heat-pressure sealed; and 7) closed, polyethylene, screw-cap bottles (15 mL) (Ampak Inc., Richmond BC), each filled with monoterpene.

In Experiment 3 and Experiments 8-14, ipsenol and ipsdienol, respectively, were released from 10-cm-lengths of C-flex® tubing (ID=1.6 mm; OD=3.2 mm) (Concept Inc., Clearwater FL), filled with ethanol solutions of either ipsenol or ipsdienol, and heat-pressure sealed at both ends. The release rate of both was approximately 0.6 mg/day at 24 °C (determined by collection of volatiles on Porapak-Q). In Experiments 1-2 and 4-7, ipsenol was released from bubble-cap lures at a rate of approximately 0.2-0.3 mg/day at 24 °C (determined by collection of volatiles on Porapak-Q).

In Experiments 15-21, verbenol was released from bubble-cap lures at a rate of approximately 1.74 mg/day at 24 °C (determined by weight loss). In Experiments 16, 17 and 21, *exo*-brevicomin was released from a 3-cm-long, glass, capillary tube (ID=13 mm; OD=15 mm) in an open, polyethylene, micro-centrifuge tube (400 µL) (Evergreen Scientific, Los Angeles CA) at a rate of approximately 0.15 mg/day at 20 °C. In Experiments 15, 18, 19 and 20, *exo*-brevicomin was released from a laminar lure at a

rate of approximately 0.1 mg/day at 24 °C (both determined by collection of volatiles on Porapak-Q).

Experiments 8-14 were conducted near Williams Lake BC, in 1988, to exploit high population levels of *I. pini*. Subsequently I found that population levels of *I. latidens* and *D. ponderosae* were low near Williams Lake and therefore conducted Experiments 1-7 and 15-21 near Princeton BC. All experiments were set in mature stands of lodgepole pine. Experiments 4-8, Chap. 2.2.2, served as checks on the responses of *I. pini* to monoterpenes near Princeton BC.

In all experiments, replicates of six 8-unit, multiple-funnel traps (Lindgren 1983) (Phero Tech Inc., Delta BC) were set in grids of 2 x 3. Replicate grids were placed at least 100 m apart, and traps were spaced 10-15 m apart within each replicate. Each trap was suspended between trees by rope such that the top funnel of each trap was 1.3-1.5 m above ground. No trap was within 2 m of any tree.

Experiments 1-7 determined the effects of 3-carene, myrcene, β-phellandrene, α-pinene, β-pinene, γ-terpinene and terpinolene, respectively, on the responses of *I. latidens* to its pheromone, ipsenol. Five replicate grids per experiment were set for Experiments 1-5 and 7 during the periods of 13 June - 19 July, 1989; 31 May - 21 June, 1989; 23 June - 22 July, 1988; 10 May - 3 June, 1989; and 9 - 28 June, 1989, respectively. Six replicate grids were set for Experiment 6 between 28 June - 19 July, 1989. Treatments, randomly assigned within each replicate, were as follows: 1) a control treatment of ipsenol alone; and 2-6) five treatments consisting of ipsenol with one monoterpene. The monoterpene treatments within a replicate differed only in release rates (Table 4). C-flex® lures of ipsenol were used in Experiment 3 while bubble-cap lures were used in the remaining six experiments.

Experiments 8-14 determined the effects of the same seven monoterpenes, respectively, on the response of *I. pini* to its pheromone, ipsdienol. Five replicate grids per experiment were set for Experiments 8-14 during the periods of 29 Aug - 7 Sept, 17 -

Table 4. Approximate release rates of monoterpenes (mg/day at 27-30 °C) used in Experiments 1-7, conducted on *I. latidens* near Princeton BC in 1988 and 1989. All traps were baited with lures releasing ipsenol at approximately 0.2-0.3 mg/day at 24 °C, unless otherwise noted.

		Treatment					
Monoterpene	Experiment	Control	1	2	3	4	5
3-Carene	1	0	0.7	14.6	34.9	184	1217
Myrcene	2	0	2.6	12.3	62.6	136	1293
β-Phellandrene	3 [a]	0	0.1	4.8	8.8	187	2084
α-Pinene	4	0	2.5	14.7	26.9	286	1239
β-Pinene	5	0	1.2	6.7	23.1	243	1199
γ-Terpinene	6	0	0.6	28.6	51.7	294	2172
Terpinolene	7	0	0.2	25.7	47.1	343	2065

[a] Release rate of ipsenol was approximately 0.6 mg/day at 24 °C.

27 Aug, 9 - 18 Aug, 31 Aug - 7 Sept, 28 Aug - 7 Sept, 27 - 29 Aug, and 7 - 18 Sept, respectively, in 1988. Treatments, randomly assigned within each replicate, were as follows: 1) a control treatment of ipsdienol alone; and 2-6) five treatments consisting of ipsdienol with one monoterpene. The monoterpene treatments within a replicate differed only in release rates (Table 5). C-flex® ipsdienol lures were used in all seven experiments.

Experiments 15-21 determined the effects of the same seven monoterpenes, respectively, on the response of *D. ponderosae* to its pheromones, *exo*-brevicomin, *cis*- and *trans*-verbenol. Five replicate grids per experiment were set for Experiments 15-21 during the periods of 25 July - 10 Aug, 1989; 14 - 24 Aug, 1988; 4 - 14 Aug, 1988; 19 July - 10 Aug, 1989; 19 July - 1 Aug, 1989; 6 Aug - 2 Sept, 1989; and 24 Aug - 1 Sept, 1988, respectively. Treatments, randomly assigned within each replicate, were as follows: 1) a control treatment consisting of *exo*-brevicomin, *cis*- and *trans*-verbenol; and 2-6) five treatments consisting of *exo*-brevicomin, *cis*- and *trans*-verbenol, with one monoterpene. The monoterpene treatments within a replicate differed only in release rates (Table 6). Bubble-cap lures of verbenol were used in all seven experiments. Micro-centrifuge tubes containing glass, capillary tubes filled with *exo*-brevicomin were used in Experiments 16, 17 and 21 while laminar lures were used in the remaining four experiments.

Subsamples of captured beetles (n=30-50) were taken at random from the lowest, highest and medial monoterpene release rates for each experiment. Sexes of *I. pini* were determined using declivital characters (Lanier and Cameron 1969), while those of *I. latidens* and *D. ponderosae* were determined by dissection and examination of genitalia.

Trap catch data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). Trap catch data, transformed by ln(Y) to remove heteroscedasticity, were subjected to two-way analysis of variance (ANOVA) using replicate and treatment as model factors. Five orthogonal contrasts were performed in

Table 5. Approximate release rates of monoterpenes (mg/day at 27-30 °C) used in Experiments 8-14, conducted on *I. pini* near Williams Lake BC inn 1988. All traps were baited with lures releasing ipsdienol at approximately 0.6 mg/day at 24 °C.

		Treatment					
Monoterpene	Experiment	Control	1	2	3	4	5
3-Carene	8	0	0.7	4.8	17.4	184	1217
Myrcene	9	0	2.6	9.4	47.0	136	646
β-Phellandrene	10	0	2.1	4.8	8.8	44	1042
α-Pinene	11	0	2.5	14.7	26.9	286	1239
β-Pinene	12	0	1.2	6.7	23.1	243	1199
γ-Terpinene	13	0	0.6	2.3	25.8	294	2172
Terpinolene	14	0	0.2	0.6	23.6	343	2065

Table 6. Approximate release rates of monoterpenes (mg/day at 27-30 °C) used in Experiments 15-21, conducted on *D. ponderosae* near Princeton BC in 1988 and 1989. All traps were baited with lures releasing *exo*-brevicomin and verbenol at approximately 0.10 and 1.74 mg/day, respectively, at 24 °C.

		Treatment					
Monoterpene	Experiment	Control	1	2	3	4	5
3-Carene	15	0	0.7	14.6	34.9	183	609
Myrcene	16 [a]	0	5.2	18.8	135.5	917	6463
β-Phellandrene	17 [a]	0	2.1	4.8	8.8	44	1042
α-Pinene	18	0	2.5	14.7	26.9	143	826
β-Pinene	19	0	1.2	6.7	23.1	121	799
γ-Terpinene	20	0	0.6	28.6	51.7	294	1086
Terpinolene	21 [a]	0	0.2	0.6	23.6	343	2065

[a] Release rate of *exo*-brevicomin was approximately 0.15 mg/day at 20 °C.

each experiment, testing the control against each monoterpene treatment within an experiment. The transformed data for each experiment were also regressed against the release rate of monoterpene, transformed by ln(X), using a general linear model. Sex ratio data from treatments 1, 3 and 5 of each experiment were analysed by Chi^2 tests of independence using the Minitab statistical package (Dept. of Statistics, Pennsylvania State University, University Park PA).

2.2.3.3 Results

Regression analyses and orthogonal contrasts successfully detected effects of monoterpenes on the attraction of *I. latidens*, *I. pini* and *D. ponderosae* to their respective pheromones. All three species showed attraction to β-phellandrene (Fig. 10). Catches of *I. latidens* in traps baited with ipsenol and the second-highest release rate of β-phellandrene were significantly greater than the control (orthogonal contrast, ANOVA, P=0.027) (Fig. 10A), consistent with results in Chap. 2.2.1. However, catches of *I. latidens* in traps baited with the highest release rate of β-phellandrene were significantly lower than the control (orthogonal contrast, ANOVA, P=0.004). Both *I. pini* and *D. ponderosae* exhibited dose-dependent attraction to β-phellandrene (Figs. 10B,C). Catches of both species were directly proportional to the release rate of β-phellandrene. Catches of *I. pini* in traps baited with treatments 2, 4 and 5 were greater than the control (orthogonal contrasts, ANOVA, P=0.003, P<0.001 and P<0.001, respectively), consistent with results in Chap. 2.2.2. None of the catches of *D. ponderosae* to traps baited with β-phellandrene was greater than the control (orthogonal contrasts, ANOVA, all P>0.18).

Two of the three species were affected by β-pinene (Fig. 11). Catches of *I. latidens* to β-pinene treatments 1-5 were independent of dose but greater than the control (orthogonal contrasts, ANOVA, P=0.066, P=0.050, P=0.086, P=0.013, and

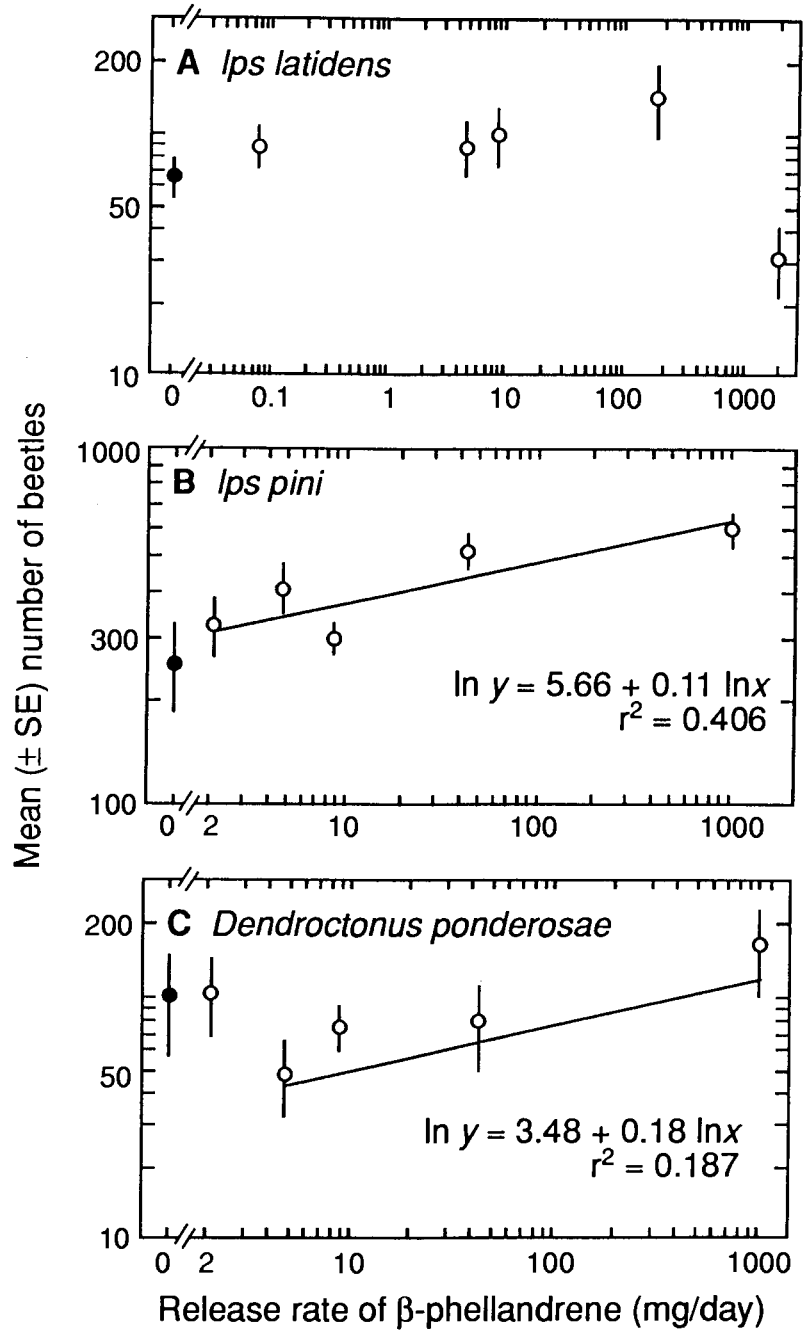


Figure 10. The effects of β-phellandrene, at various release rates, on the attraction of *I. latidens* (A), *I. pini* (B), and *D. ponderosae* (C) to multiple-funnel traps baited with their respective pheromones: ipsenol, ipsdienol, verbenol and *exo*-brevicomin (n=5, 5, and 5, respectively). The slopes of the regression lines for *I. pini* and *D. ponderosae* are significantly different from zero (t test, $P<0.001$ and $P=0.057$, respectively).

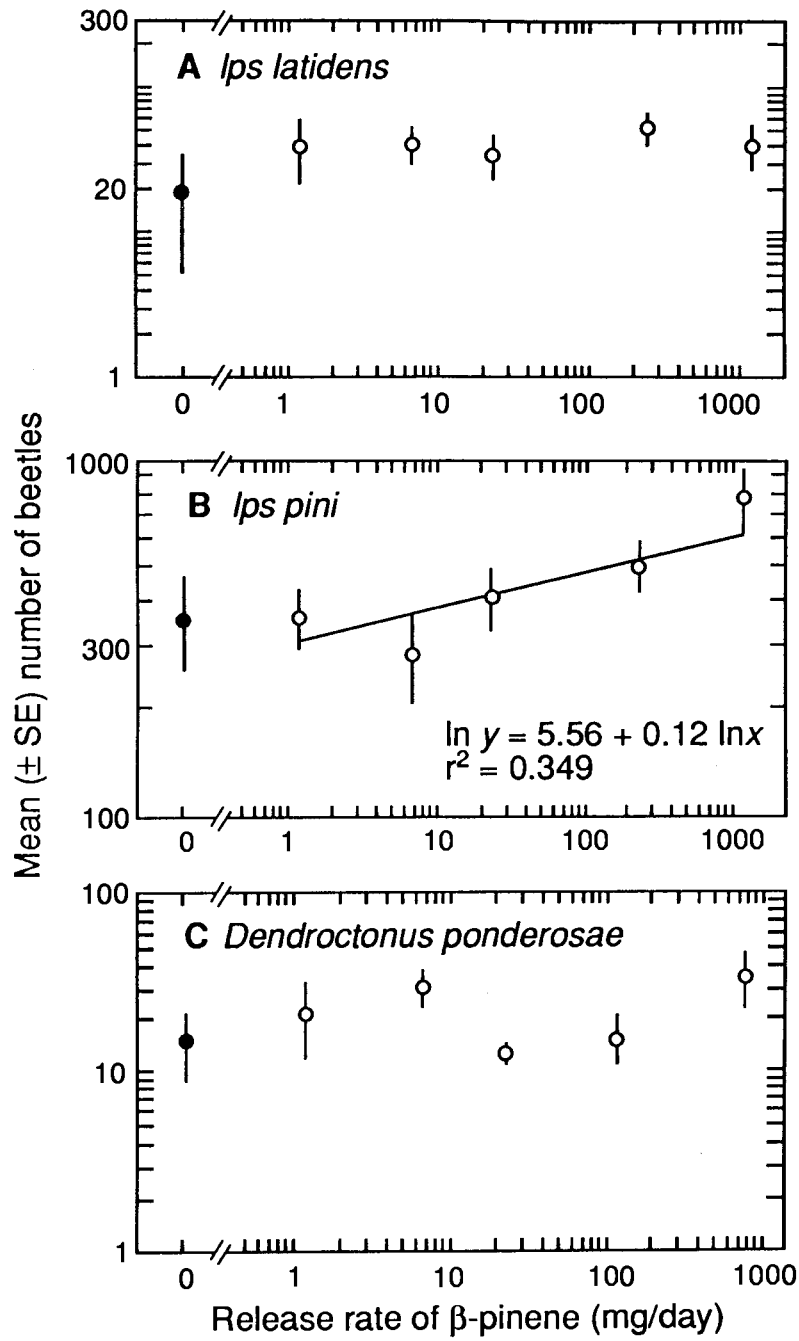


Figure 11. The effects of β-pinene, at various release rates, on the attraction of *I. latidens* (A), *I. pini* (B), and *D. ponderosae* (C) to multiple-funnel traps baited with their respective pheromones: ipsenol, ipsdienol, verbenol and *exo*-brevicomin (n=5, 5, and 3, respectively). The slope of the regression line for *I. pini* is significantly different from zero (t test, P=0.002).

P=0.040, respectively) (Fig. 11A). *Ips pini* exhibited dose-dependent attraction to β-pinene (Fig. 11B). Catches in traps baited with β-pinene at the two highest release rates (treatments 4 and 5) were significantly greater than the control (orthogonal contrasts, ANOVA, P=0.024 and P<0.001, respectively). The presence of β-pinene had no effect on the responses of *D. ponderosae* (Fig. 11C) (orthogonal contrasts, ANOVA, all P>0.18). One replicate was excluded from the analysis because only 12 beetles were caught.

All three species showed dose-dependent responses to 3-carene and myrcene (Figs. 12 and 13). Catches of *I. latidens* were inversely proportional to the release rate of 3-carene (Fig. 12A), while those of *I. pini* and *D. ponderosae* were directly proportional (Figs. 12B,C). Catches of *I. latidens* in traps baited with 3-carene at the highest release rates were significantly lower than the control (orthogonal contrast, ANOVA, P=0.002). Relative to controls, traps baited with 3-carene at the two highest release rates (treatments 4 and 5) caught significantly more *I. pini* (orthogonal contrast, ANOVA, P<0.001 and P<0.001), consistent with results in Chap. 2.2.2, and *D. ponderosae* (orthogonal contrast, ANOVA, P=0.086 and P=0.040, respectively).

The trap catches of both *I. latidens* and *I. pini* were inversely proportional to the release rate of myrcene (Fig. 13A,B), while those of *D. ponderosae* were directly proportional (Fig. 13C). Relative to controls, traps baited with myrcene at the three highest release rates (treatments 3, 4 and 5) caught significantly fewer *I. latidens* (orthogonal contrasts, ANOVA, P=0.035, P=0.010, and P=0.017, respectively) and *I. pini* (orthogonal contrasts, ANOVA, P=0.002, P=0.005, and P<0.001, respectively). Catches of *D. ponderosae* were significantly higher in traps baited with myrcene at the two highest release rates (treatments 4 and 5) than in control traps (orthogonal contrasts, ANOVA, P=0.042 and P=0.002, respectively).

None of the species showed dose-dependent responses to γ-terpinene (Fig. 14), although γ-terpinene did affect the behavioral responses of two species. Catches of

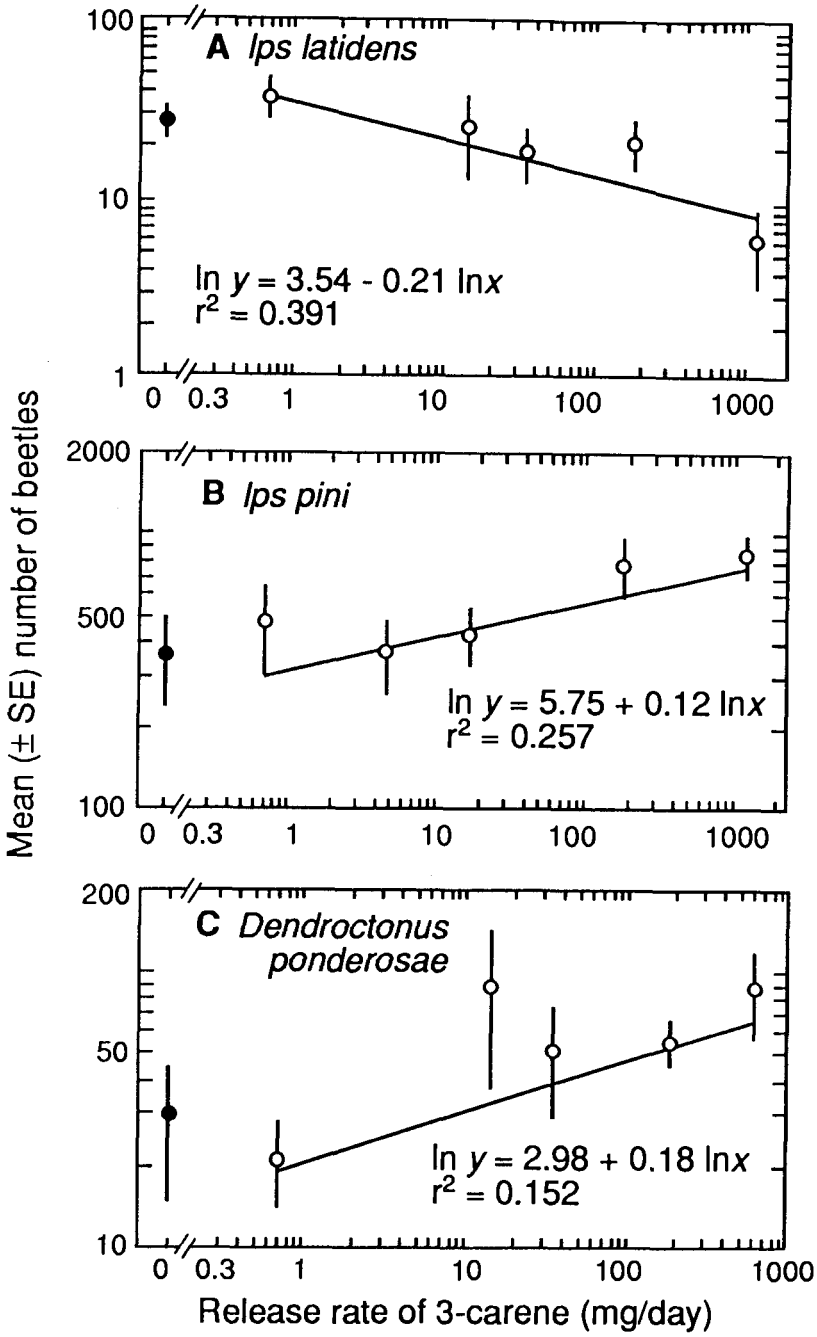


Figure 12. The effects of 3-carene, at various release rates, on the attraction of *I. latidens* (A), *I. pini* (B), and *D. ponderosae* (C) to multiple-funnel traps baited with their respective pheromones: ipsenol, ipsdienol, verbenol and *exo*-brevicomin (n=4, 5, and 5, respectively). The slopes of the regression lines for *I. latidens*, *I. pini* and *D. ponderosae* are significantly different from zero (t test, P=0.003, P=0.010, and P=0.054, respectively).

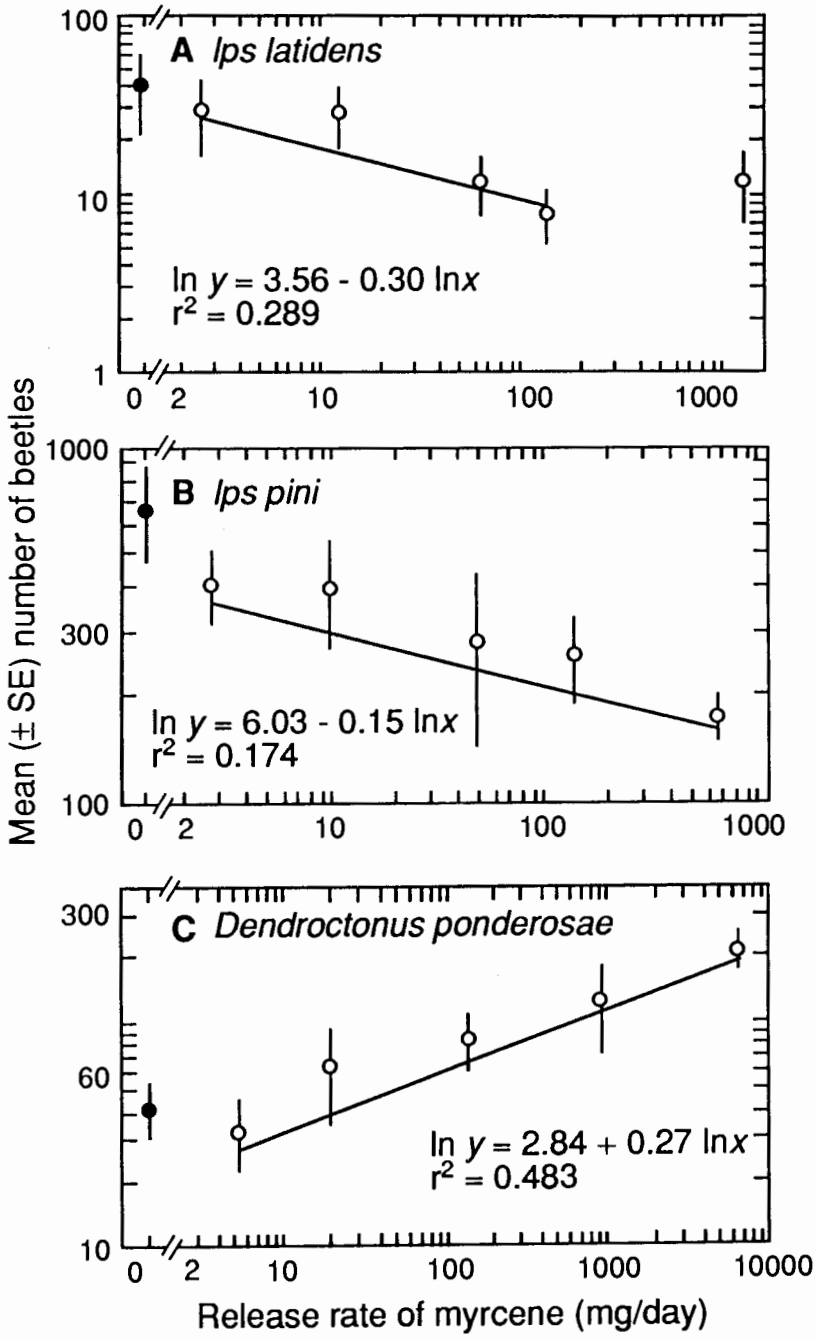


Figure 13. The effects of myrcene, at various release rates, on the attraction of *I. latidens* (A), *I. pini* (B), and *D. ponderosae* (C) to multiple-funnel traps baited with their respective pheromones: ipsenol, ipsdienol, verbenol and *exo*-brevicomin (n=4, 5, and 5, respectively). The slopes of the regression lines for *I. latidens*, *I. pini* and *D. ponderosae* are significantly different from zero (t test, P=0.032, P=0.038, and P<0.001, respectively).

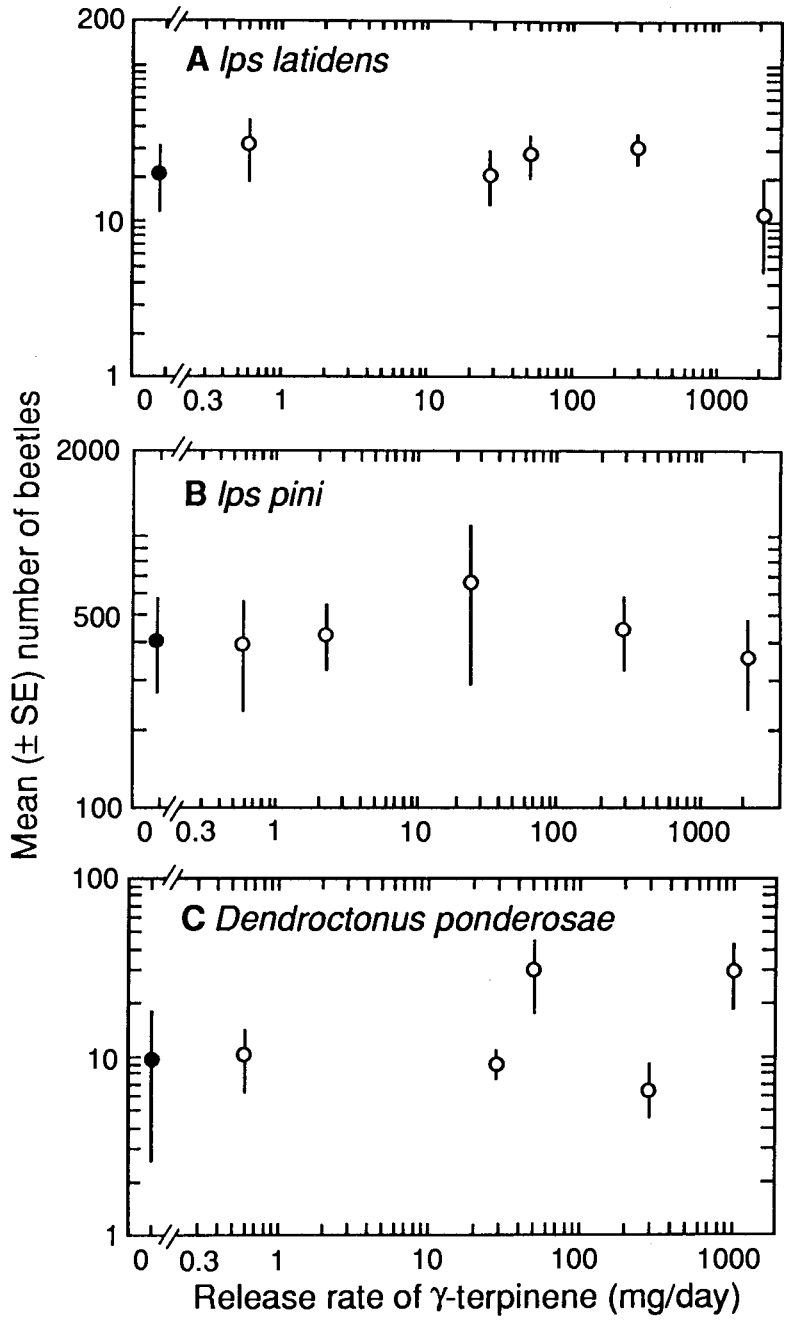


Figure 14. The effects of γ-terpinene, at various release rates, on the attraction of *I. latidens* (A), *I. pini* (B), and *D. ponderosae* (C) to multiple-funnel traps baited with their respective pheromones: ipsenol, ipsdienol, verbenol and *exo*-brevicomin (n=6, 5, and 3, respectively).

I. latidens in traps baited with γ-terpinene at the highest release rate were lower than those in controls (orthogonal contrast, ANOVA, P=0.086). Catches of *D. ponderosae* in traps baited with γ-terpinene at two of the three highest release rates (treatments 3 and 5) were significantly greater than those of controls (orthogonal contrasts, ANOVA, P=0.032 and P=0.028, respectively). γ-Terpinene had no effect on the attraction of *I. pini* (orthogonal contrasts, ANOVA, all P>0.21).

Terpinolene significantly reduced trap catches of all three species (Fig. 15). Catches of *I. latidens* and *I. pini* were inversely proportional to the release rate of terpinolene (Figs. 15A,B). Catches of *I. pini* in traps baited with terpinolene at the two highest release rates (treatments 4 and 5) were significantly lower than those in controls (orthogonal contrasts, ANOVA, P<0.001 and P<0.001, respectively), consistent with results in Chap. 2.2.2. Reductions in catches of *I. latidens* were not detectable by orthogonal contrast. However, treatment 2 (at the second-lowest release rate) resulted in significantly greater trap catches of *I. latidens* than the control (orthogonal contrast, ANOVA, P=0.040). Catches of *D. ponderosae* were significantly lower in traps baited with terpinolene at the highest release rate than those in controls (orthogonal contrast, ANOVA, P=0.047).

There was no evidence of proportional differences in catches of *I. latidens*, *I. pini*, and *D. ponderosae*, relative to proportional differences in the release rate of α-pinene (Fig. 16). Catches of *I. latidens* in traps baited with α-pinene at the second-lowest release rate (treatment 2) were lower than those in control traps (orthogonal contrast, ANOVA, P=0.024). Catches of *I. pini* in traps baited at the third-highest release rate (treatment 3) were significantly lower than those in control traps (orthogonal contrast, ANOVA, P<0.001).

Evidence of sex-specific responses of the three species to different release rates of monoterpenes was found in only two experiments. The proportion of male *I. pini* caught in traps baited with β-phellandrene increased as the release rate of β-phellandrene

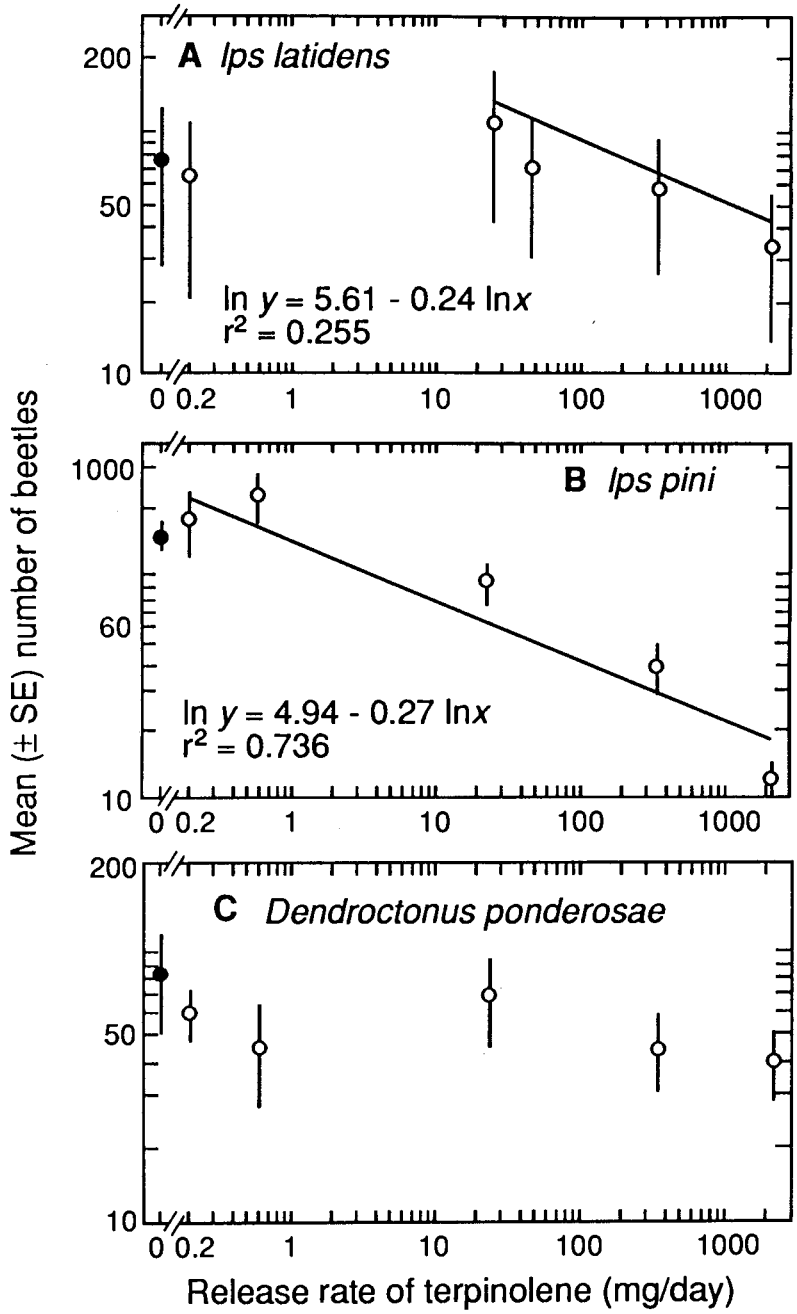


Figure 15. The effects of terpinolene, at various release rates, on the attraction of *I. latidens* (A), *I. pini* (B), and *D. ponderosae* (C) to multiple-funnel traps baited with their respective pheromones: ipsenol, ipsdienol, verbenol and *exo*-brevicomin (n=5, 5, and 5, respectively). The slopes of the regression lines for *I. latidens* and *I. pini* are significantly different from zero (t test, P=0.094 and P<0.001, respectively).

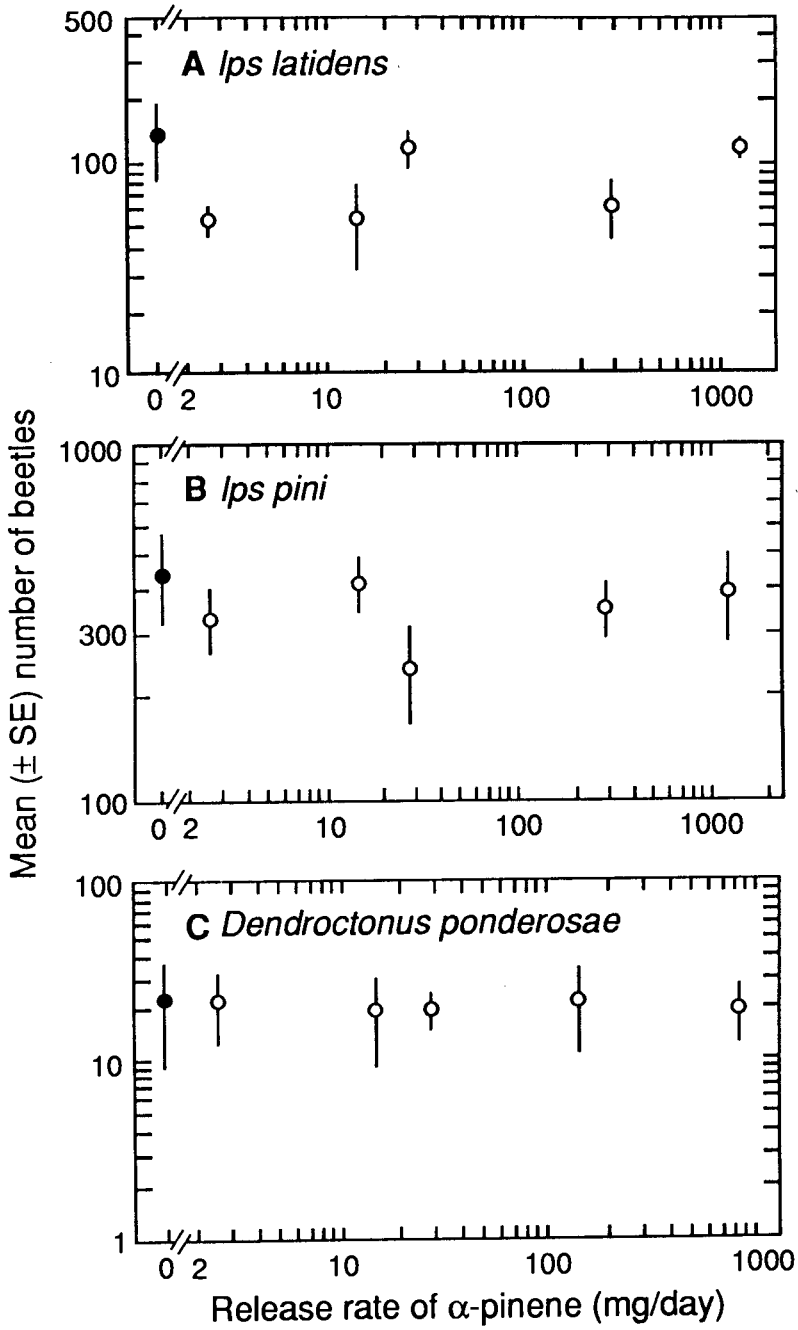


Figure 16. The effects of α-pinene, at various release rates, on the attraction of *I. latidens* (A), *I. pini* (B), and *D. ponderosae* (C) to multiple-funnel traps baited with their respective pheromones: ipsenol, ipsdienol, verbenol and *exo*-brevicomin (n=4, 5, and 5, respectively).

increased (Chi^2 test of association, df=2, $P<0.025$). The proportion of male *D. ponderosae* in traps baited with γ-terpinene decreased as the release rate of γ-terpinene increased (Chi^2 test of association, df=2, $P<0.01$). The mean (±SE) proportion of male *I. latidens* and *I. pini*, in experiments lacking any association between sex ratio and release rates of monoterpenes, were both 0.20 ± 0.015; significantly different from 0.5 for both species (t tests, df= 6 and 5, respectively, $P<0.001$ for both), as in Chaps. 2.1, 2.2.1 and 2.2.2. The mean (±SE) proportion of male *D. ponderosae* caught in experiments lacking any association between sex ratio and release rate of monoterpenes was 0.51 ± 0.035; not significantly different from 0.5 (t test, df=5, $P=0.82$), as in Chap. 2.1.

2.2.3.4 Discussion

The pheromone-mediated responses of *I. latidens*, *I. pini* and *D. ponderosae* in stands of lodgepole pine were significantly affected by six of seven monoterpenes commonly found in the phloem tissue of lodgepole pine (Figs. 10-16). Most monoterpenes increased or decreased attraction; some did both. The most common type of response was a dose-dependent one, as evidenced by significant regressions with slopes differing significantly from zero. *Ips latidens* showed dose-dependent inhibition to ipsenol by three of the six monoterpenes (Figs. 12A, 13A and 15A). *Ips pini* showed dose-dependent attraction to three of the seven monoterpenes tested with ipsdienol (Figs. 10B, 11B and 12B) and dose-dependent inhibition to ipsdienol by two monoterpenes (Figs. 13B and 15B). *Dendroctonus ponderosae* showed dose-dependent attraction to three monoterpenes when tested with *exo*-brevicomin and a verbenol mix (Figs. 10C, 12C and 13C). The attraction of *D. ponderosae* to 3-carene and myrcene (Figs. 12C and 13C) is consistent with Billings et al. (1976), Conn et al. (1983) and Borden et al. (1987b). Conn et al. (1983) found that β-phellandrene had no effect on *D. ponderosae*.

However, they employed low release rates (7 mg/day), comparable to my lowest release rate which showed little effect.

It is possible that in cases where orthogonal contrasts disclosed attraction or inhibition, but regression analyses failed to detect dose-dependent relationships, that the appropriate range was not adequately sampled, i.e., the dose-dependency may occur over a small range of release rates. In a clear example of the lack of dose-dependency, *I. latidens* showed a preference for traps baited with β-pinene at any release rate (Fig. 11A). Such a response may be indicative of a kairomone that serves to identify suitable host species.

A dose-dependent function of responses to monoterpene kairomones may be typical of bark beetles. Such a range of responses may relate to a broad range of suitable host material rather than one very specific type. Habitats used by bark beetles tend to be patchy and ephemeral (Atkins 1968; Alcock 1982). Beetles may not have the luxury of waiting for the perfect host. Even when a host is found, the optimal areas for breeding may already be taken by conspecifics, thereby forcing the newcomer into suboptimal phloem conditions.

Some evidence of multifunctionality in the responses of beetles to monoterpenes was apparent. β-Phellandrene at the highest release rate inhibited the response of *I. latidens* to ipsenol-baited traps while at the second-highest release rate it resulted in trap catches greater than those in controls. In contrast, trap catches of *D. ponderosae* were reduced with the second-lowest release rate of β-phellandrene but then increased in direct proportion to the release rate of β-phellandrene. *Dendroctonus ponderosae* may prefer hosts releasing large amounts of β-phellandrene while *I. latidens* may prefer hosts releasing only low amounts of β-phellandrene. This hypothesis is consistent with the observation that *I. latidens* seems to prefer relatively-drier, phloem tissue than *D. ponderosae* (Miller and Borden 1985) while *D. ponderosae* attacks mature lodgepole pines (Safranyik et al. 1974) that produce copious amounts of resin (Shrimpton 1978;

Cates and Alexander 1982; Raffa and Berryman 1987), and presumably copious amounts of β-phellandrene as well.

Dose-dependent relationships and multifunctionality suggest that beetles may be able to make determinations of blend compositions. Such an ability would facilitate greater host discrimination on the part of beetles. An experimental design wherein the release rates of two or more monoterpenes are varied would be the best way to determine if ratio determinations are being made by beetles.

My results demonstrate that *I. latidens*, *I. pini* and *D. ponderosae* can achieve some degree of premating, reproductive isolation through their responses to monoterpenes (Table 7). In particular the combination of myrcene, 3-carene and high levels of β-phellandrene could separate the three species in areas of sympatry such as the Shinnish Creek watershed near Princeton BC; the other responses may serve as 'insurance'. Species-specificity in the use of kairomones also suggests that beetles respond best to stimuli that convey information regarding the presence of host qualities for which they have a competitive edge. If so then single-species aggregations of beetles may be a consequence of interspecific competition rather than a mechanism for reproductive isolation.

Table 7. Summary of the kairomonal effects of seven monoterpenes on the attraction of three sympatric species of bark beetles to their respective pheromones in stands of lodgepole pine. No effect (O), attraction (+) and repulsion (-) were determined by regression analyses and orthogonal contrasts at P=0.05 (unless otherwise noted) in Experiments 1-21.

Monoterpene	*I. latidens*	*I. pini*	*D. ponderosae*
α-Pinene	O	O	O
γ-Terpinene	− [a]	O	+
Myrcene	−	−	+
Terpinolene	±	−	−
3-Carene	−	+	+
β-Phellandrene	±	+	+
β-Pinene	+	+	O

[a] Significant at P=0.086.

2.3 SPECIES-SPECIFIC PHEROMONES

Pheromones are semiochemicals that convey information between conspecifics (Bethe 1932; Karlson and Butenandt 1959; Karlson and Lüscher 1959; Kalmus 1965; Law and Regnier 1971). Natural selection has favored: 1) individuals that produced pheromones that affected the behavior of conspecifics; and 2) individuals that modified their behavior in response to pheromones produced by conspecifics (Burghardt 1970; Law and Regnier 1971; Whittaker and Feeny 1971; Nordlund and Lewis 1976; Shorey 1977; Nordlund 1981; Dicke and Sabelis 1988). Pheromones are used to convey signals or messages (i.e. to communicate); a function that should be beneficial for both participants (Burghardt 1970; Law and Regnier 1971; Otte 1974; Rutowski 1981). In other words, pheromones can be defined with respect to their function, rather than their mechanisms, as semiochemicals used in intraspecific communication.

Reproduction is an intraspecific event. Intraspecific communication should be closely associated with the facilitation of reproductive isolation; more so than acts of exploitative, interspecific information transfer such as in the use of kairomones. The use of species-specific pheromones should result in attraction of only conspecifics. Moreover the traits for production and response can be associated genetically.

Species-specificity in the use of pheromones is common in insects (Wood 1970; Lanier and Burkholder 1974; Roelofs and Cardé 1974; Shorey 1976; Silverstein 1977; Birch 1978; Borden 1982; Roelofs and Brown 1982; S.L. Wood 1982; Cardé and Baker 1984; West-Eberhard 1984; Baker 1986; Byers 1989a,b; Linn and Roelofs 1989). Specificity can be achieved by one or more of the following: 1) species-specific pheromones; 2) species-specific doses of one pheromone; 3) species-specific combinations of several pheromones; and 4) species-specific ratios of several pheromones. Each subsequent mode of specificity is more complex than the previous one, with respect to

the amount of information that can be encoded, and should be evidenced by behavioral responses that are increasingly more sensitive to precise semiochemical characteristics.

Ips latidens, *I. pini* and *Dendroctonus ponderosae* show species-specificity in the use of semiochemicals, specifically in the use of species-specific kairomone blends (Chaps. 2.2.1, 2.2.2 and 2.2.3). As well, species-specificity in the use of pheromones seems to occur through the use of species-specific pheromones rather than species-specific doses or blends. *Ips pini* uses both enantiomers of ipsdienol as pheromones (Stewart 1975; Plummer et al. 1976; Birch et al. 1980a) while *D. ponderosae* uses (-)-*trans*-verbenol (Pitman et al. 1968; Pitman 1971; Ryker and Rudinsky 1982; Libbey et al. 1985; Borden et al. 1987b; Pierce et al. 1987) and both enantiomers of *exo*-brevicomin (Pitman et al. 1969; Rudinsky et al. 1974; Pitman et al. 1978; Borden et al. 1983; Libbey et al. 1985; Borden et al. 1987b). The pheromone for *I. latidens* is not known.

2.3.1 Ipsenol: An aggregation pheromone for *Ips latidens*

2.3.1.1 Introduction

Various scolytid species show behavioral responses to different enantiomers of ipsenol and the related chiral alcohol, ipsdienol (Borden 1982). In California, *Ips latidens* were caught, *albeit* in low numbers, on traps baited with either ipsenol or a mixture of ipsenol and *cis*-verbenol (Wood et al. 1967). In Idaho, *I. latidens* were attracted to sources of racemic ipsenol, alone, and in combination with bolts of ponderosa pine (Furniss and Livingston 1979). However, since the production of ipsenol by *I. latidens* was not determined in either study, the question of ipsenol as a pheromone for *I. latidens* is still unresolved.

My objective was to determine the identity of the pheromone(s) used by *I. latidens*. I tested the three following hypotheses: 1) one or both sexes of *I. latidens*

would produce one or both enantiomers of ipsenol and/or one or both enantiomers of *cis*-verbenol; 2) *I. latidens* would be attracted to chiral ipsenol; and 3) *cis*-verbenol would act synergistically in increasing attraction of *I. latidens* to chiral ipsenol.

2.3.1.2 Materials and Methods

In 1984, adult *I. latidens* were obtained from a 2-yr-old colony, originating near the east gate of Manning Park BC. Using the gelatin-pill-capsule technique (Borden 1967), 16 adult males and 5 adult females were restrained, individually, on non-infested bolts of lodgepole pine, collected near Princeton BC. They were allowed to bore into the bark and feed for 24 hrs. The frass of each individual was crushed in 150 μL of pentane. These extracts were analysed by splitless, capillary, gas chromatography (Hewlett Packard HP 5890 using a 30-m x 0.25-mm ID fused silica column). The identities and integrities of ipsenol and *cis*-verbenol were verified by mass spectrometry using splitless, capillary, gas chromatography (Hewlett Packard HP 5985B).

(±)-Ipsenol (chemical purity, 98%) was obtained from Bedoukian Research Inc. (Danbury CT). B.J. Johnson (Dept. of Chemistry, Simon Fraser University) supplied chiral ipsenols (optical purities, 96% (-) and 94% (+), respectively; chemical purities, 98%). Phero Tech Inc.(Delta BC) supplied polyethylene, bubble-cap lures containing the following chemicals: 1) (±)-ipsenol (chemical purity, 98%) in solution with 1,3-butanediol; 2) 1,3-butanediol (chemical purity,>99%); and 3) chiral *cis*-verbenols (optical purities, 84% (-) and 94% (+), respectively ; chemical purities, 98%). (-)-β-Phellandrene (chemical purity, 98%) was obtained from H.D. Pierce, Jr. (Dept. of Chemistry, Simon Fraser University).

β-Phellandrene was released from closed, polyethylene, micro-centrifuge tubes (1.8 mL)(Evergreen Scientific, Los Angeles CA) at a rate of approximately 8 mg/day at 27-30 °C (determined by weight loss). Ipsenol lures consisted of either 10-cm lengths of

C-flex® tubing (ID=1.6 mm; OD= 2.4 mm) (Concept Inc., Clearwater FL) filled with a solution of ipsenol in ethanol, or polyethylene, bubble-caps filled with a solution of ipsenol in 1,3-butanediol, and heat-pressure sealed. The release rates of ipsenol from these devices were approximately 0.6 and 0.2-0.3 mg/day, respectively, at 24 °C (determined by collection of volatiles on Porapak-Q). Ethanol lures consisted of 10-cm lengths of C-flex® tubing, each filled with ethanol and heat-pressure sealed. The release rate of ethanol from these devices was approximately 5-6 mg/day at 24 °C (determined by weight loss). *cis*-Verbenol was released from polyethylene, bubble-cap lures at a rate of 3-6 mg/day at 27-30 °C (determined by weight loss).

In all experiments, 8-unit, multiple-funnel traps (Lindgren 1983) (Phero Tech Inc., Delta BC) were set in mature stands of lodgepole pine near Princeton BC. Each trap was suspended such that the top funnel of each trap was 1.3-1.5 m above ground. No trap was within 2 m of any tree. Treatments were randomly assigned within replicates. Sexes of captured *I. latidens* were determined by dissection and examination of genitalia.

In Experiments 1-2, replicate grids were placed at least 100 m apart, and traps were spaced 10-15 m apart within each replicate. The effect of chiral ipsenol was tested in Experiment 1. Eleven replicates of six traps per replicate, were set in grids of 2 x 3, from 23 May to 2 July, 1987. The treatments, using C-flex® lures, were as follows: 1) blank control; 2) ethanol control; 3) (±)-ipsenol (0.6 mg/day); 4) (±)-ipsenol (1.2 mg/day); 5) (-)-ipsenol (0.6 mg/day); and 6) (+)-ipsenol (0.6 mg/day).

Experiment 2 tested for interaction between (-)-*cis*-verbenol and the combination of (±)-ipsenol and β-phellandrene. β-Phellandrene is used as a kairomone by *I. latidens* (Chap. 2.2.1). Nine replicates of four traps per replicate, were set in grids of 2 x 2, from 21 May to 23 June, 1988. The treatments, using C-flex® lures, were as follows: 1) ethanol control; 2) (±)-ipsenol with β-phellandrene; 3) (-)-*cis*-verbenol; and 4) the combination of (±)-ipsenol, β-phellandrene and (-)-*cis*-verbenol.

Experiment 3 tested for interaction between (+)-*cis*-verbenol and (±)-ipsenol. β-phellandrene was not used due to lack of availability. Traps were placed 50 m apart in a single, large grid pattern measuring 200 x 400 m. Ten replicate blocks of four linearly-consecutive traps per block, were set from 21 June to 10 July, 1989. The treatments, using bubble-cap lures, were as follows:
1) 1,3-butanediol control; 2) (±)-ipsenol; 3) (+)-*cis*-verbenol; and 4) (±)-ipsenol and (+)-*cis*-verbenol.

The data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). Trap catches of *I. latidens* were transformed by ln(Y+1) to remove heteroscedasticity while sex ratio data were normalised by an arcsin transformation. Homoscedastic data were subjected to either one-, two- or three-way analysis of variance (ANOVA). The model factors were replicate and treatment in Experiment 1 while replicate, *cis*-verbenol, ipsenol, and the interaction between *cis*-verbenol and ipsenol, were used in Experiment 2. In Experiment 3, a full-factorial two-way ANOVA was employed. Two orthogonal contrasts were performed in Experiment 1 while Duncan's Multiple Range tests were used in Experiments 2-3 when $P<0.05$.

2.3.1.3 Results

Ipsenol was found in the frass of 10 of 16 male *I. latidens* (estimated range, 10 ng to 1 μg), but not in the frass of any female. The chirality of ipsenol was not determined because we were unable to separate the acetyl lactate diastereomers (Slessor et al. 1985) of synthetic (±)-ipsenol by gas chromatography. *cis*-Verbenol was not found in any samples. The major monoterpene in the frass was β-phellandrene. β-Phellandrene is the major monoterpene in the phloem tissue of lodgepole pine (Mirov 1961; Shrimpton 1972,1973) and acts as a kairomone for *I. latidens* (Chap. 2.2.1).

In Experiment 1, *I. latidens* were significantly attracted to chiral ipsenol, with a preference for (-)-ipsenol (Fig. 17A). Since there is no significant interaction between ethanol and (±)-ipsenol (Chap. 2.3.4), the results in Experiment 1 may be attributed solely to ipsenol. The sex ratios of *I. latidens* caught in traps baited with ipsenol were affected by chirality [ANOVA, F(3,30), P=0.048]. Proportionally more males responded to (-)-ipsenol than to either (±)- or (+)-ipsenol (Fig. 17B).

cis-Verbenol significantly reduced the attraction of *I. latidens*. In Experiments 2-3, (-)- and (+)-*cis*-verbenol significantly inhibited attraction of *I. latidens* to traps baited with (±)-ipsenol, ethanol and β-phellandrene, and (±)-ipsenol and ethanol, respectively (Figs. 18-19).

2.3.1.4 Discussion

I and Seybold et al. (1991) have demonstrated that ipsenol is an aggregation pheromone for *I. latidens*. Ipsenol is produced by males and attracts both males and females, in the laboratory (Seybold et al. 1991) and in the field (Fig. 17). Our combined results agree with the field data of Furniss and Livingston (1979) and in part with that of Wood et al. (1967) . In contrast to results from California (Wood et al. 1967), both enantiomers of *cis*-verbenol inhibited the response of *I. latidens* (Figs. 18-19). *cis*-Verbenol was not produced by *I. latidens*.

Reproductive isolation is possible among *I. latidens*, *I. pini* and *D. ponderosae* in stands of lodgepole pine through the use of species-specific pheromones. The pheromones of *I. latidens* and *I. pini* are the enantiomers of ipsenol (Fig. 17; Seybold et al. 1990) and ipsdienol (Stewart 1975; Plummer et al. 1976; Birch et al. 1980a; Chaps. 3.1.1-3.1.2), respectively, while those of *D. ponderosae* are (-)-*trans*-verbenol (Pitman et al. 1968; Pitman 1971; Ryker and Rudinsky 1982; Libbey et al. 1985; Borden et al. 1987b; Pierce et al. 1987) and both enantiomers of *exo*-brevicomin (Pitman et al. 1969;

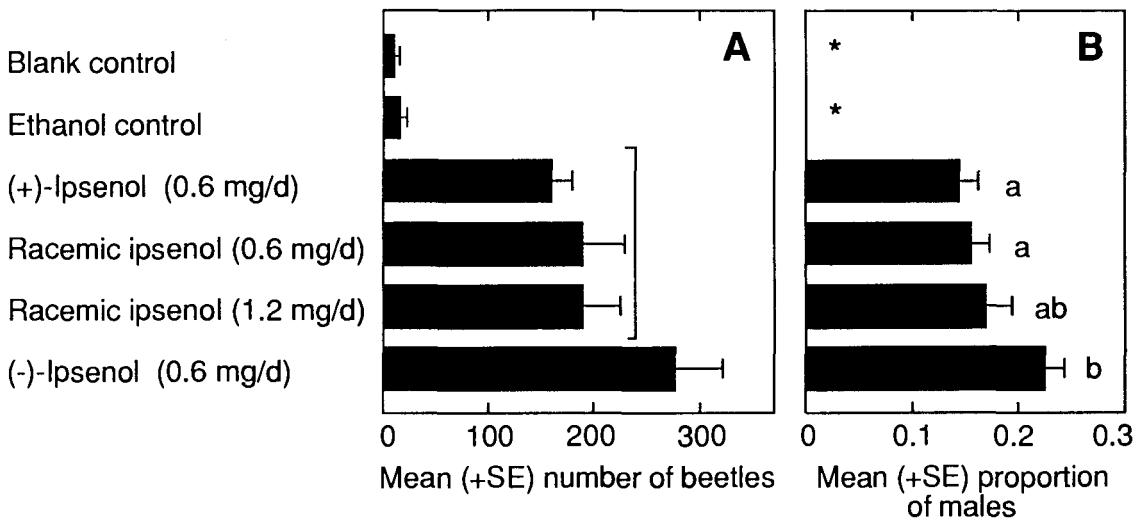


Figure 17. The effect of chiral ipsenol on the number (A) and sex ratio (B) of *I. latidens* captured in multiple-funnel traps near Princeton BC, from 23 May to 2 July, 1987 (n=11). Means grouped by a line are significantly different from the blank and ethanol controls, and (-)-ipsenol [orthogonal contrasts, $F(1,49)$, $P<0.001$ and $P=0.025$, respectively, on data transformed by $\ln(Y+1)$]. Mean proportions of males followed by the same letter are not significantly different at $P=0.05$ [Duncan's Multiple Range test on data transformed by $\arcsin(Y)$].

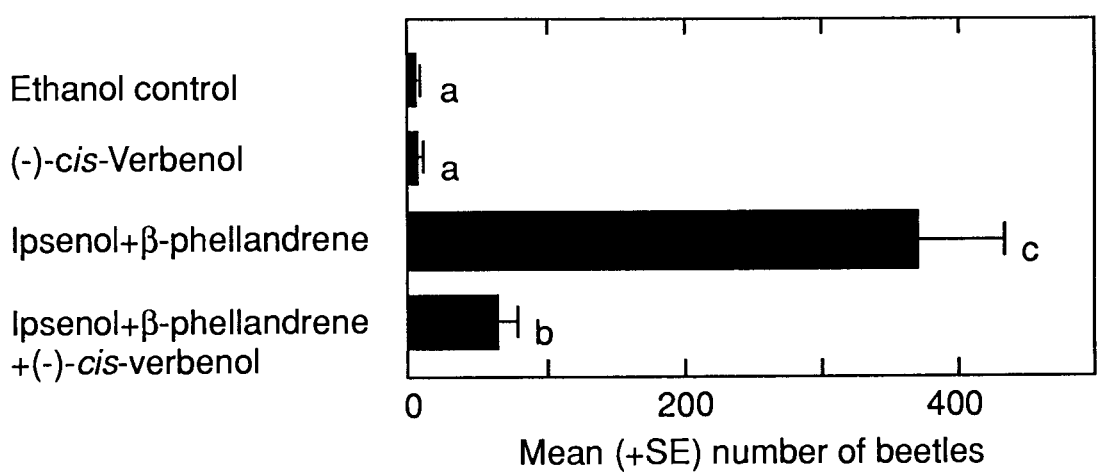


Figure 18. The effect of (-)-*cis*-verbenol and the combination of ipsenol and β-phellandrene on the attraction of *I. latidens* to multiple-funnel traps near Princeton BC from 8 to 23 June, 1988 (n=9). Means followed by the same letter are not significantly different at P=0.05 [Duncan's Multiple Range test on data transformed by ln(Y+1)].

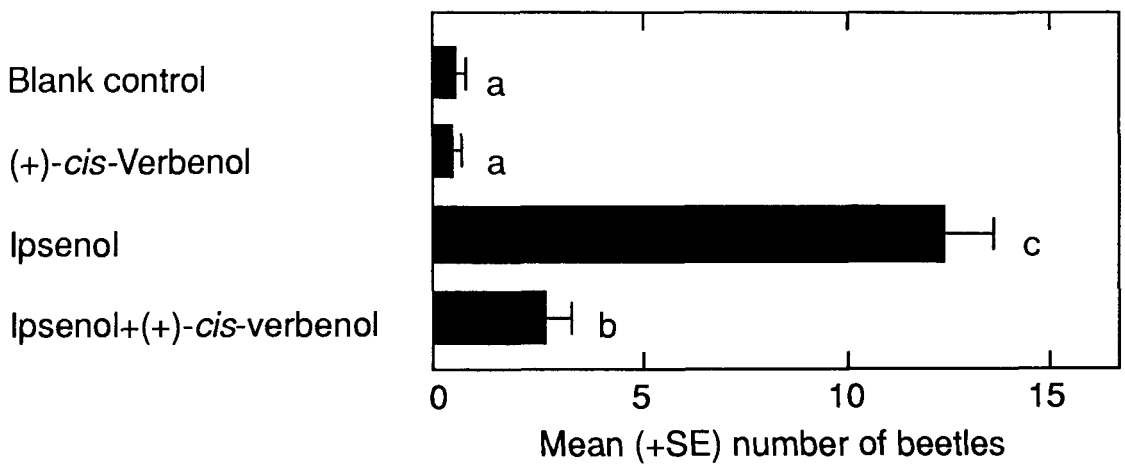


Figure 19. The effect of (+)-*cis*-verbenol and ipsenol on the attraction of *I. latidens* to multiple-funnel traps near Princeton BC from 21 June to 10 July, 1989 (n=10). Means followed by the same letter are not significantly different at P=0.05 [Duncan's Multiple Range test on data transformed by ln(Y+1)].

Rudinsky et al. 1974; Pitman et al. 1978; Borden et al. 1983; Libbey et al. 1985; Borden et al. 1987b).

However, the use of species-specific pheromones by these three species cannot be used as support for the hypothesis that all species use unique pheromones to maintain reproductive isolation. The choice of species for my studies was biased by *a priori* knowledge that at least two species were known to use different pheromones. In addition there was circumstantial evidence suggesting that the pheromone for *I. latidens* was different from the two other species. Other species of bark beetles show considerable overlap in the use of pheromones. Eight of ten species of *Dendroctonus*, studied to date, use frontalin as a pheromone (Werner et al. 1981; Borden 1982; Conn et al. 1983; Payne et al. 1987). Of 17 species of *Ips* studied, males of 16 species produce ipsdienol while males of 11 species produce ipsenol; males of eight species produce both ipsenol and ipsdienol (Vité et al. 1972; Francke et al. 1980; Borden 1982; Kohnle et al. 1988). Geographic separation does play a role in maintaining reproductive isolation among some of these species. However the use of the same pheromones is apparent even among sympatric species breeding in the same species of host. Of three *Ips* species that breed in loblolly pine, all three produce verbenols (Vité et al. 1972) and two use ipsdienol as a pheromone (Borden 1982; Vité et al. 1972). The bark beetles, *I. mexicanus*, *I. emarginatus* and *Pityogenes plagiatus knechteli*, breed in lodgepole pine in British Columbia (Bright 1976) and are attracted to the pheromones of *I. latidens* and *I. pini* (Miller and Borden 1990; unpublished data). In general, the use of species-specific pheromones may make a contribution towards maintaining reproductive isolation and minimising interspecific competition for hosts but is unlikely to be the sole mechanism.

2.3.2 *cis*-Verbenol: An aggregation pheromone for *Dendroctonus ponderosae*

2.3.2.1 Introduction

Various studies on the use of semiochemicals by *Dendroctonus ponderosae*, including some that were specifically aimed at determining the effect of *trans*-verbenol, used *trans*-verbenol contaminated with 6-20 % of *cis*-verbenol (Pitman 1971; Billings et al. 1976; Ryker and Rudinsky 1982; Borden et al. 1983; Conn et al. 1983). The role of *trans*-verbenol as a pheromone for *D. ponderosae* has subsequently been verified with chemical purities >97% (Ryker and Rudinsky 1982; Libbey et al. 1985; Borden et al. 1987b). However no study has attempted to discern the role of *cis*-verbenol in the chemical ecology of *D. ponderosae*. Both *cis*- and *trans*-verbenols are produced by female *D. ponderosae* (Pitman et al. 1969; Hughes 1973b; Ryker and Rudinsky 1982; Libbey et al. 1985; Pierce et al. 1987; Hunt et al. 1989). Antennae of both sexes of *D. ponderosae* are sensitive to *cis*- and *trans*-verbenol equally (Whitehead 1986; Whitehead et al. 1989). It is likely, therefore, that *cis*-verbenol is a pheromone for *D. ponderosae*.

Interpretation of studies using contaminated *trans*-verbenol must be suspect until the role of *cis*-verbenol can be ascertained. Moreover this information could have significant commercial implications. Semiochemical-based manipulation of *D. ponderosae* has become a major component of lodgepole pine silviculture in British Columbia (Borden and Lacey 1985). The tree bait most commonly used against *D. ponderosae* uses an 87:13 mix of *trans*- and *cis*-verbenol (PheroTech Inc., Delta BC), together with myrcene and *exo*-brevicomin. *Dendroctonus ponderosae* uses myrcene as a kairomone (Billings et al. 1976; Conn et al. 1983; Libbey et al. 1985; Borden et al. 1987b) and both enantiomers of *exo*-brevicomin as male-produced pheromones (Pitman et al. 1969; Rudinsky et al. 1974; Pitman et al. 1978; Borden et al. 1983; Libbey et al. 1985; Borden et al. 1987b).

My objective was to demonstrate that *cis*-verbenol is an aggregation pheromone for *D. ponderosae* in stands of lodgepole pine. I tested the two following hypotheses: 1) *cis*-verbenol would increase attraction of *D. ponderosae* to traps baited with myrcene and *exo*-brevicomin; and 2) *cis*- and *trans*-verbenol would have an additive effect on the attraction of *D. ponderosae*.

2.3.2.2 Materials and Methods

PheroTech Inc. (Delta BC) supplied the following lures: 1) (±)-*exo*-brevicomin (chemical purity, 98%) laminar lures ; 2) polyethylene, bubble-caps containing *cis*-verbenol (chemical purity, 96%) in solution with 1,3-butanediol; and 3) polyethylene, bubble-caps containing *trans*-verbenol (chemical purity, 99%). The release rates of *exo*-brevicomin, *cis*- and *trans*-verbenol were approximately 0.10, 2.58 and 2.58, respectively, at 24 °C (determined by collection of volatiles on Porapak-Q for *exo*-brevicomin and by weight loss for the verbenols). The chiral ratios of *cis*- and *trans*-verbenol were both 83% *S*-(-): 17% *R*-(+). β-Myrcene (chemical purity, 98%) was obtained from PheroTech Inc. (Delta BC) and released from closed, polyethylene, screw-cap bottles (15 mL) (Ampak Inc., Richmond BC) at a rate of approximately 281 mg/day at 24 °C (determined by weight loss).

Forty, 8-unit, multiple-funnel traps (Lindgren 1983) (Phero Tech Inc., Delta BC) were set in 10 replicate grids of 2 x 2 in stands of mature lodgepole pine near Princeton BC. Grids were spaced at least 100 m apart, and traps were set 10-15 m apart within each replicate. Each trap was suspended between trees by rope such that the top funnel of each trap was 1.3-1.5 m above ground. No trap was within 2 m of any tree. All grids were set during the period of 2 to 26 Sept, 1989. Treatments were randomly assigned within each replicate. The control treatment consisted of myrcene and *exo*-brevicomin while the remainder consisted of myrcene, *exo*-brevicomin and one of the following: 1)

cis-verbenol; 2) *trans*-verbenol; 3) *cis*- and *trans*-verbenol. Sexes of captured *D. ponderosae* were determined by dissection and internal examination of genitalia.

The data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). Trap catch data were transformed by ln(Y+1) to remove heteroscedasticity while sex ratio data were normalised and homoscedasticised by $\arcsin\sqrt{Y}$. Homoscedastic data were subjected to 3-way analysis of variance (ANOVA), using replicate, *cis*-verbenol, *trans*-verbenol, and the interaction of *cis*- and *trans*-verbenol, as model factors. Duncan's Multiple Range tests were performed when $P<0.05$.

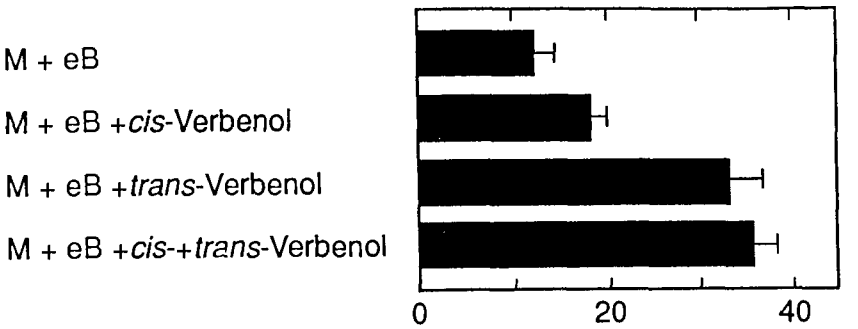
2.3.2.3 Results and Discussion

Both *cis*- and *trans*-verbenol significantly increased the catches of *D. ponderosae* to traps baited with myrcene and *exo*-brevicomin (Fig. 20A). There was no significant interaction between *cis*- and *trans*-verbenol on trap catches; the effect was additive.

In contrast, *cis*- and *trans*-verbenol had differing effects on the sex ratio of *D. ponderosae* caught in traps (Fig. 20B). Proportionally more males responded to traps baited with *trans*-verbenol, *exo*-brevicomin and myrcene than to traps baited with *exo*-brevicomin and myrcene alone. However this effect was reduced when *cis*-verbenol was present with *trans*-verbenol.

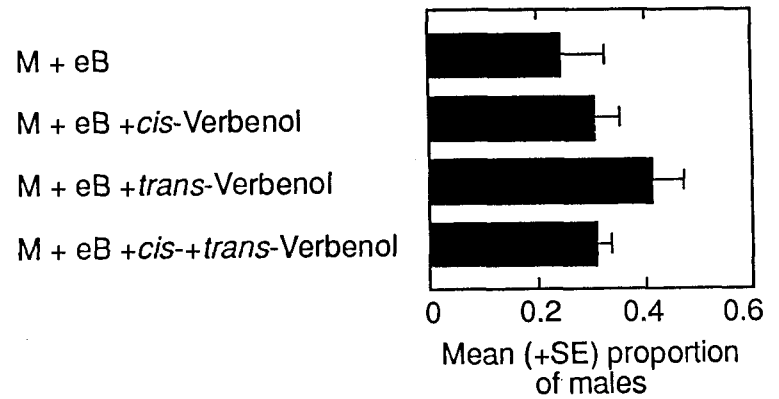
My results indicate that *cis*-verbenol is an aggregation pheromone for *D. ponderosae*. It is produced by female *D. ponderosae* (Pitman et al. 1969; Hughes 1973b;Ryker and Rudinsky 1982; Libbey et al. 1985; Pierce et al. 1987) and is attractive to both sexes (Fig. 20A,B). Interpretations of results from previous studies that used *trans*-verbenol, with chemical purities less than 97%, should consider the effect of *cis*-verbenol, and the possible interactions of *cis*-verbenol with other treatments.

The effect of the interaction between *cis*- and *trans*-verbenol on the sex-specific



ANOVA

Source	df	P
A	9	<0.001
B	1	0.045
C	1	<0.001
B*C	1	0.185
Error	27	



ANOVA

Source	df	P
A	1	0.872
B	1	0.091
A*B	1	0.039
Error	35	

Figure 20. The effects of *cis*- and *trans*-verbenol on the number (A) and sex ratio (B) of *D. ponderosae* captured in multiple-funnel traps baited with myrcene (M) and *exo*-brevicomin (eB) near Princeton BC from 2 to 26 Sept, 1989 (n=10). Number (A) and sex ratio data (B) were transformed by $\ln(Y+1)$ and $\arcsin\sqrt{Y}$, respectively. Replicate (A), *cis*-verbenol (B), *trans*-verbenol (C) and B*C were the model factors in A while *cis*-verbenol (A), *trans*-verbenol (B) and A*B were the factors in B.

responses suggests that males and females differ in their specificity to verbenol ratios. The equilibrium ratio of *trans*- and *cis*-verbenol following oxidation of α-pinene is approximately 87:13 and may be the key to male response. Fortunately this verbenol ratio is currently employed operationally for controlling *D. ponderosae* (PheroTech Inc., Delta BC).

2.3.3 Dose-dependent responses of *Ips latidens*, *I. pini* and *Dendroctonus ponderosae* to their respective pheromones

2.3.3.1 Introduction

In general, bark beetles do not seem to use single, species-specific pheromones (Vité et al. 1972; Francke et al. 1980; Werner et al. 1981; Borden 1982; Conn et al. 1983; Payne et al. 1987; Kohnle et al. 1988). If pheromones serve a role in maintaining reproductive and ecological isolation, then they must be used in a fashion that can encode a more complex message. One way would be to base specificity on the release rates of a single pheromone from either individuals or aggregations. Individuals of species that are generally large in size and breed in nutrient-rich phloem tissue may produce larger amounts of pheromone per individual relative to individuals of species that are generally small in size and breed in drier material. As well, the production of pheromone could be related to the number of individuals producing pheromone. A group of conspecifics of a species that aggregates in large numbers probably produces a greater amount of pheromone relative to a group of conspecifics of a species that aggregates in small numbers. It is likely that if both occur then they are probably correlated to each other as well.

Dose-dependent response is the sequential increase or decrease in responses by individuals as the release rate of a semiochemical increases. Dose-dependent response is not the attraction of more individuals due simply to the fact that higher release rates

result in larger areas of influence for the pheromone. These two separate phenomena have been confounded in the past and tests of dose response should assay the treatments on the same number of individuals. In order to achieve this situation in field experiments, I placed traps within a replicate quite close together (10-15 m apart). The flight range of these beetles is at least several hundred meters and in no case was there evidence of beetles within the confines of a replicate. I assumed that the plumes originating from the different treatments within a replicate coalesce to form a single large plume, thereby ensuring that the same pool of beetles was tested for preferences. Dose-dependent response differs from an all-or-none response where no response occurs until a threshold concentration is reached and the response is maximal and constant thereafter.

The attraction of males of some lepidopteran species to their respective female-produced pheromones or pheromone blends increases with an increase in release rate even when the area of influence is controlled by having all treatments in close proximity (Maitlen et al. 1976; Flint et al. 1978; Kamm and McDonough 1979; McNally and Barnes 1980; Bellas and Bartell 1983). In several species, attraction reaches a maximum at some intermediate release rate and further increase in release rate results in inhibition (Maitlen et al. 1976; Kamm and McDonough 1979; McNally and Barnes 1980; Mitchell et al. 1988). Evidence of a similar dose-dependent specificity in response to pheromones has not been demonstrated for Scolytidae, due primarily to a lack of attempts to test for dose-dependency..

My objectives were twofold. Firstly, I planned to verify the identities of the pheromones for *I. latidens*, *I. pini* and *D. ponderosae* in stands of lodgepole pine in British Columbia. Secondly, I wanted to determine if and how pheromones released at different rates affect the responses of beetles. I tested the five following hypotheses: 1) *I. pini* would be attracted to (±)-ipsdienol in a dose-dependent fashion; 2) *I. latidens* would be attracted to (±)-ipsenol in a dose-dependent fashion; 3) the dose-dependent

response for *I. latidens* would also occur in the presence of the kairomone, β-phellandrene; 4) attraction of *D. ponderosae* to a blend of myrcene and *exo*-brevicomin would be increased by a mix of *cis*- and *trans*-verbenol in a dose-dependent fashion; and 5) attraction of *D. ponderosae* to a blend of myrcene, *cis*- and *trans*-verbenol would be increased by *exo*-brevicomin in a dose-dependent fashion.

2.3.3.2 Methods and Materials

β-Myrcene, (±)-ipsenol, (±)-ipsdienol, (±)-*exo*-brevicomin, and a 13:87 mix of *cis*- and *trans*-verbenol (chemical purities, all 98%) were obtained from PheroTech Inc. (Delta BC). The chiral ratios of *cis*- and *trans*-verbenol were both 83% (-): 17% (+). (-)-β-Phellandrene (chemical purity, 98%) was obtained from H.D. Pierce, Jr. (Dept. of Chemistry, Simon Fraser University). PheroTech Inc. (Delta BC) supplied the following lures: 1) (±)-*exo*-brevicomin (chemical purity, 98%) laminar lures; 2) (±)-*exo*-brevicomin (chemical purity, 98%) capillary-tube lures; and 3) polyethylene, bubble-cap lures containing a 13:87 mix of *cis*- and *trans*-verbenol [chemical purity, 98%; chiral ratios, both 83% *S*-(-): 17% *R*-(+)]. The release rates from these three types of lures were approximately 0.10, 0.21 and 2.06 mg/day at 24 °C (determined by collection of volatiles on Porapak-Q).

β-Phellandrene was released from closed, polyethylene, micro-centrifuge tubes (1.8 mL) (Evergreen Scientific, Los Angeles CA) at a rate of approximately 8 mg/day at 27-30 °C (determined by weight loss). Myrcene was released from closed, polyethylene, screw-cap bottles (15 mL) (Ampak Inc., Richmond BC) at a rate of approximately 281 mg/day at 24 °C (determined by weight loss).

In Experiment 1 and Experiments 2-3, ipsdienol and ipsenol, respectively, were released from lengths (10-100 cm) of C-flex® tubing (ID=1.6 mm; OD=3.2 mm)

(Concept Inc., Clearwater FL), filled with ethanol solutions of either ipsdienol or ipsenol, respectively, and heat-pressure sealed at both ends.

In Experiments 4-5, *cis*- and *trans*-verbenol were released together from the following devices: 1-2) open, polypropylene, micro-centrifuge tubes (1.5 mL) (Quality Scientific Plastics, Petaluma CA) containing either one or five 2-cm-long, glass, capillary tubes (ID=1.5 mm; OD=1.8 mm), each sealed at one end and filled with verbenols; and 3) bubble-cap lures.

In Experiments 6-7, *exo*-brevicomin was released from the following devices: 1) open, polypropylene, micro-centrifuge tubes (1.5 mL) (Quality Scientific Plastics, Petaluma CA) each containing one Microcap® disposable pipette (2 μL) (Drummond Scientific Co., Broomall PA), sealed at one end and filled with *exo*-brevicomin; 2-4) laminar lures (0.81, 2.42, or 6.45 cm^2); and 5) closed, polyethylene, micro-centrifuge tubes (0.25 mL) (Evergreen Scientific, Los Angeles CA), each filled with *exo*-brevicomin.

In all experiments, grids of 8-unit, multiple-funnel traps (Lindgren 1983) (PheroTech Inc., Delta BC) were set in mature stands of lodgepole pine. Replicate grids were placed at least 100 m apart, and traps were spaced 10-15 m apart within each replicate. Each trap was suspended between trees such that the top funnel of each trap was 1.3-1.5 m above ground. No trap was within 2 m of any tree. Experiment 1 was conducted near Williams Lake BC, to exploit high population levels of *I. pini*. Subsequently I found that population levels of *I. latidens* and *D. ponderosae* were low near Williams Lake and therefore conducted Experiments 2-7 near Princeton BC.

Experiment 1 determined the effects of different release rates of ipsdienol on the attraction of *I. pini*. Five replicate grids (2 x 3) were set between 17 July and 16 Aug, 1988. The treatments, randomly assigned within each replicate. were as follows: 1) blank control; and 2-6) five ipsdienol treatments, differing only in release rates (Table 8).

Experiments 2 and 3 determined the effects of different release rates of ipsenol on the attraction of *I. latidens*, with and without β-phellandrene. Five replicate grids

Table 8. Approximate release rates (μg/day at 24 °C, unless otherwise stated) of kairomones and pheromones used in Experiments 1-7, conducted in stands of lodgepole pine in British Columbia in 1988 and 1989.

		Treatment					
	Experiment	Control	1	2	3	4	5
Ipsdienol	1	0	60	180	600	1,800	6,000
Ipsenol	2	0	30	90	300	900	3,000
Ipsenol [a]	3	0	30	90	300	900	3,000
Verbenol mix [b]	4	0	120	230	1,150	5,170	25,830
Verbenol mix [c]	5	0	230	2,580	12,910	–	–
exo-Brevicomin [d]	6	0	110	210	840	2,060	12,340
exo-Brevicomin [d]	7	0	10	40	100	–	–

[a] With β-phellandrene released at approximately 8 mg/day at 27-30 °C.

[b] With myrcene and *exo*-brevicomin released at approximately 281 and 0.21 mg/day, respectively.

[c] With myrcene and *exo*-brevicomin released at approximately 281 and 0.10 mg/day , respectively.

[d] With myrcene and verbenol mix released at approximately 281 and 2.58 mg/day, respectively.

(2 x 3) per experiment were set for Experiments 2 and 3 during the periods of 23 June to 10 July and 10 to 20 July, 1988, respectively. The treatments for Experiment 2, randomly assigned within each replicate, were as follows: 1) blank control; and 2-6) five ipsenol treatments, differing only in release rates (Table 8). The treatments for Experiment 3 were similar to those for Experiment 2 and included β-phellandrene in all traps (Table 8).

Experiments 4 and 5 determined the effects of different release rates of verbenol on the attraction of *D. ponderosae* to traps baited with *exo*-brevicomin and myrcene. The release rates of *exo*-brevicomin in 1988 and 1989 were approximately 0.21 and 0.10 mg/day, respectively, at 24 °C (determined by collection of volatiles on Porapak-Q). Five and ten replicate grids (2 x 3 and 2 x 2, respectively) were set for Experiments 4 and 5, respectively, during the periods of 14 to 24 Aug, 1988, and 20 Aug to 26 Sept, 1989, respectively. The treatments in Experiment 4, randomly assigned within each replicate, were as follows: 1) *exo*-brevicomin and myrcene; and 2-6) *exo*-brevicomin, myrcene and one of five verbenol treatments, differing only in release rates (Table 8). The treatments in Experiment 5 were similar to those in Experiment 4 except that I used only three verbenol treatments (Table 8).

Experiments 6-7 determined the effects of different release rates of *exo*-brevicomin on the attraction of *D. ponderosae* to traps baited with *cis*- and *trans*-verbenol, and myrcene. The release rates of *cis*- and *trans*-verbenol were approximately 0.34 and 2.24 mg/day, respectively, at 24 °C (determined by weight loss). The treatments for Experiment 6, randomly assigned within each replicate, were as follows: 1) *cis*- and *trans*-verbenol, and myrcene; and 2-6) *cis*- and *trans*-verbenol, myrcene and one of five *exo*-brevicomin treatments, differing only in release rates (Table 8). The treatments in Experiment 7 were similar to those in Experiment 6 except that I used only three *exo*-brevicomin treatments in Experiment 7 (Table 8).

In Experiment 1, sex ratios of captured *I. pini* were determined for all catches. In Experiments 2-7, subsamples of captured beetles (n=30-50) were taken at random from the lowest, medial and highest pheromone release rates for each experiment. Sexes of captured *I. pini* were determined using declivital characters (Lanier and Cameron 1969) while those of captured *I. latidens* and *D. ponderosae* were determined by dissection and examination of genitalia.

Trap catch data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). Trap catch data were transformed by ln(Y+1) in Experiments 1-3, and ln(Y) in Experiments 4-7, to remove heteroscedasticity. Sex ratio data in Experiment 1 were normalised by arcsin(Y). Homoscedastic data were subjected to two-way analysis of variance (ANOVA) using replicate and treatment as model factors. Five orthogonal contrasts were performed in each of Experiments 1-4, and 6, while three were performed in each of Experiments 5 and 7, comparing the control against each pheromone treatment within an experiment. Trap catch data, transformed by ln(Y), from each experiment, and untransformed sex ratio data from Experiment 1, were regressed against the release rate of pheromone, transformed by ln(X), using treatment as the only factor in a general linear model. Sex ratio data from Experiments 2-7 were analysed by Chi^2 tests of independence/association using the Minitab statistical package ver. 5.1.1 (Dept. of Statistics, Pennsylvania State University, University Park PA).

2.3.3.3 Results

Regression analyses and orthogonal contrasts successfully verified the use of ipsdienol and ipsenol as pheromones by *I. pini* and *I. latidens*, respectively. *Ips pini* exhibited dose-dependent attraction to ipsdienol (Fig. 21A), with significantly higher catches in all traps baited with ipsdienol than in controls (orthogonal contrasts, ANOVA, all $P<0.015$). The male proportion of *I. pini* captured in ipsdienol-baited traps decreased as

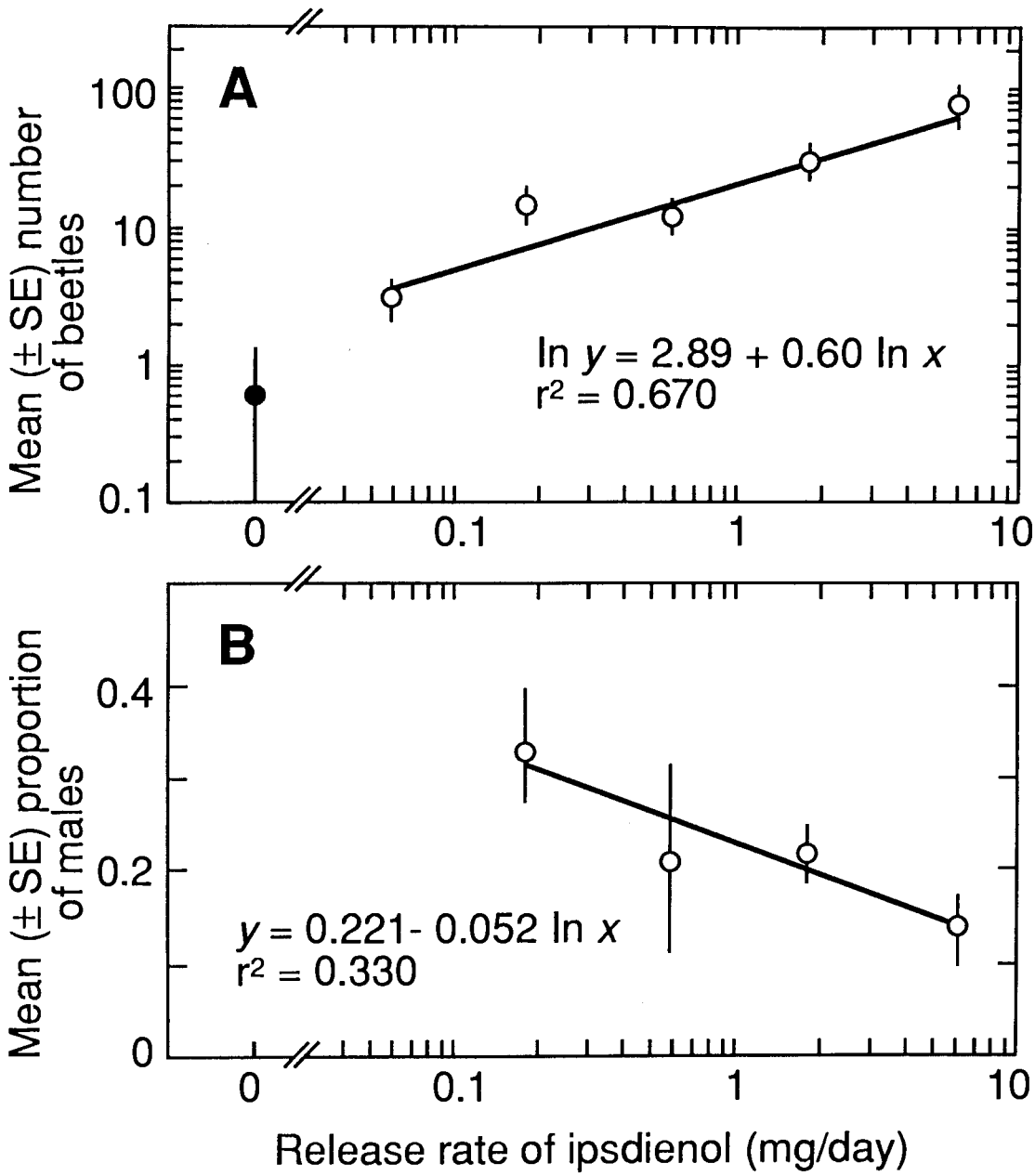


Figure 21. The effects of ipsdienol, released at various rates, on the number (A) and sex ratio (B) of *I. pini* captured in multiple-funnel traps near Williams Lake BC from 17 July to 16 Aug, 1988 (n=5). The slopes of the regression lines are significantly different from zero (t test, $P<0.001$ and $P=0.020$, respectively).

the release rate increased (Fig. 21B). Females showed a stronger dose-dependent response to ipsdienol than males.

In contrast, *I. latidens* did not exhibit a dose-dependent to ipsenol (Figs. 22A,B). Significantly more beetles were caught in ipsenol-baited traps than in controls in both experiments (orthogonal contrasts, ANOVA, all $P<0.001$). *Ips latidens* showed a reduction in attraction at the highest release rate in Experiment 2 when compared to the remaining four ipsenol treatments as a group (orthogonal contrast, ANOVA, $P=0.049$). Inhibition was not detected in Experiment 3 (orthogonal contrast, ANOVA, $P=0.375$). There was no significant change in sex ratio among trap catches to the ipsenol treatments (Chi^2 test, df=2 for both, $P>0.05$ for both).

Results of Experiments 4-5 on the capture of *D. ponderosae* were confounded by contamination of the high-release verbenol lures by the antiaggregation pheromone, verbenone (2-20% of residual material) (Fig. 23A,B). *Dendroctonus ponderosae* showed dose-dependent inhibition to verbenone. In Experiment 4, treatments with the second and third lowest release rates caught significantly more beetles than the control (orthogonal contrasts, ANOVA, both $P=0.036$). There was no change is sex ratio among trap catches to the verbenol treatments (Chi^2 test, both df=2, both $P>0.05$).

In no instance did *exo*-brevicomin increase the attraction of *D. ponderosae* to traps baited with myrcene and a verbenol mix (Figs. 24A,B). High-release rates of *exo*-brevicomin resulted in dose-dependent inhibition (Fig. 24B). There was no change in sex ratio among trap catches to *exo*-brevicomin (Chi^2 test, both df=2, both $P>0.05$).

2.3.3.4 Discussion

My results verified that ipsenol, ipsdienol and verbenol are pheromones for *I. latidens*, *I. pini* and *D. ponderosae*, respectively. I was unable to verify that *exo*-brevicomin is a pheromone for *D. ponderosae*. Borden et al. (1987b) found that at a release rate of 0.05

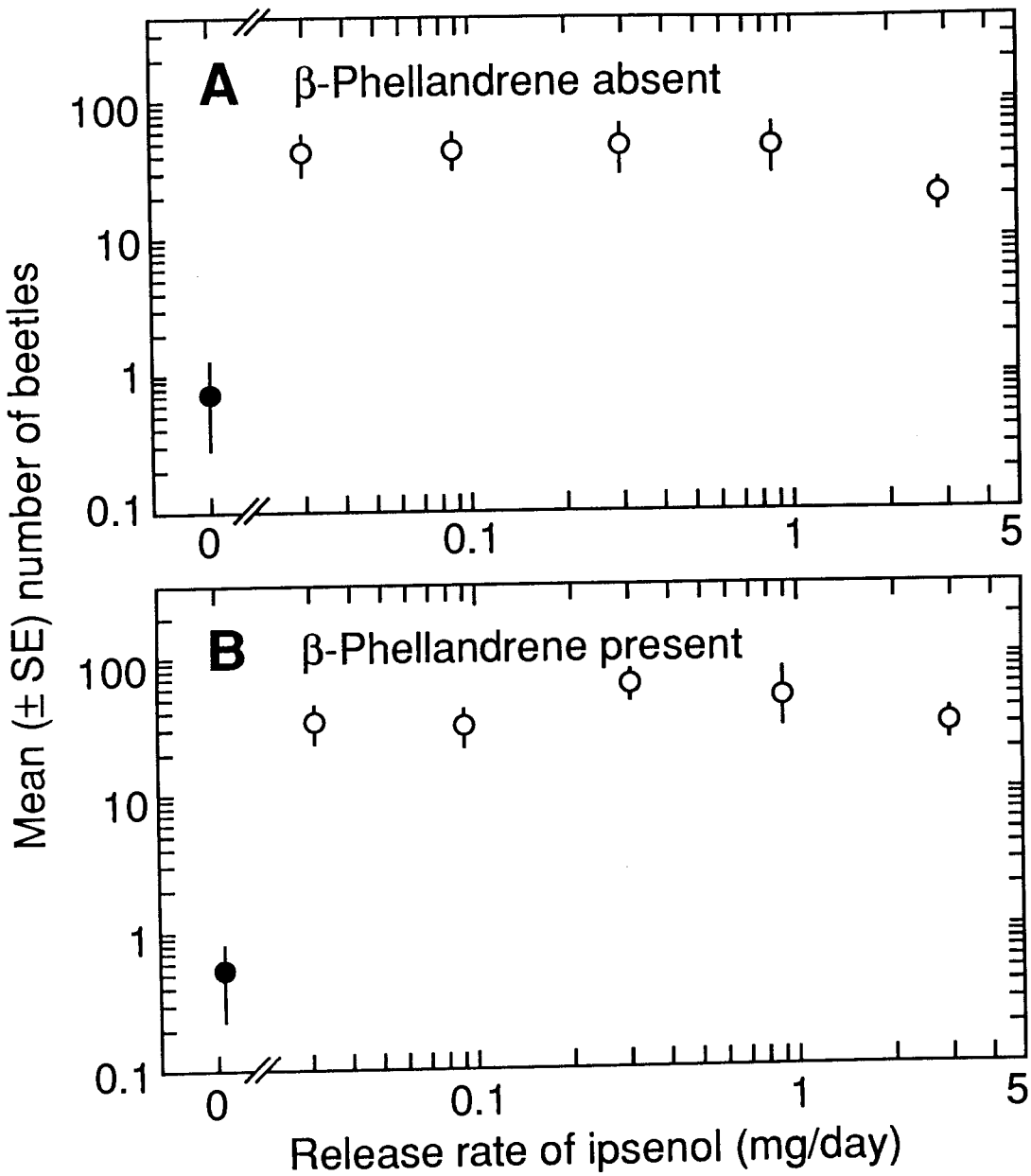


Figure 22. The effects of ipsenol, released at various rates without (A) and with (B) β-phellandrene, on the attraction of *I. latidens* to multiple-funnel traps near Princeton BC in 1988.

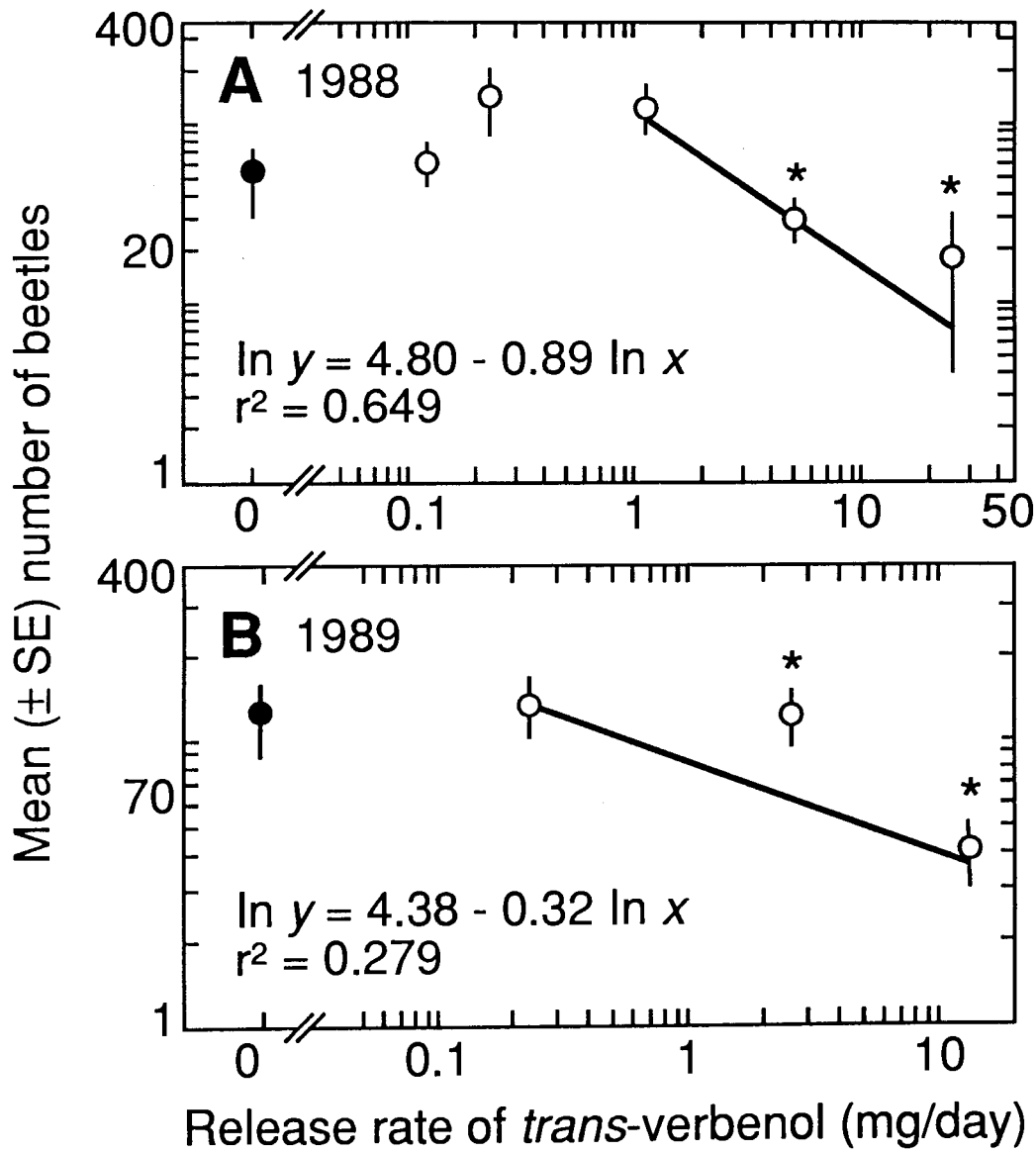


Figure 23. The effects of a 13:87 mix of *cis*- and *trans*-verbenol, released at various rates, on the attraction of *D. ponderosae* to multiple-funnel traps baited with myrcene and *exo*-brevicomin near Princeton BC in 1988 (A) and 1989 (B). Slopes of regression lines are significantly different from zero (t test, P=0.002 and P=0.003, respectively). Some treatments (*) were contaminated with verbenone.

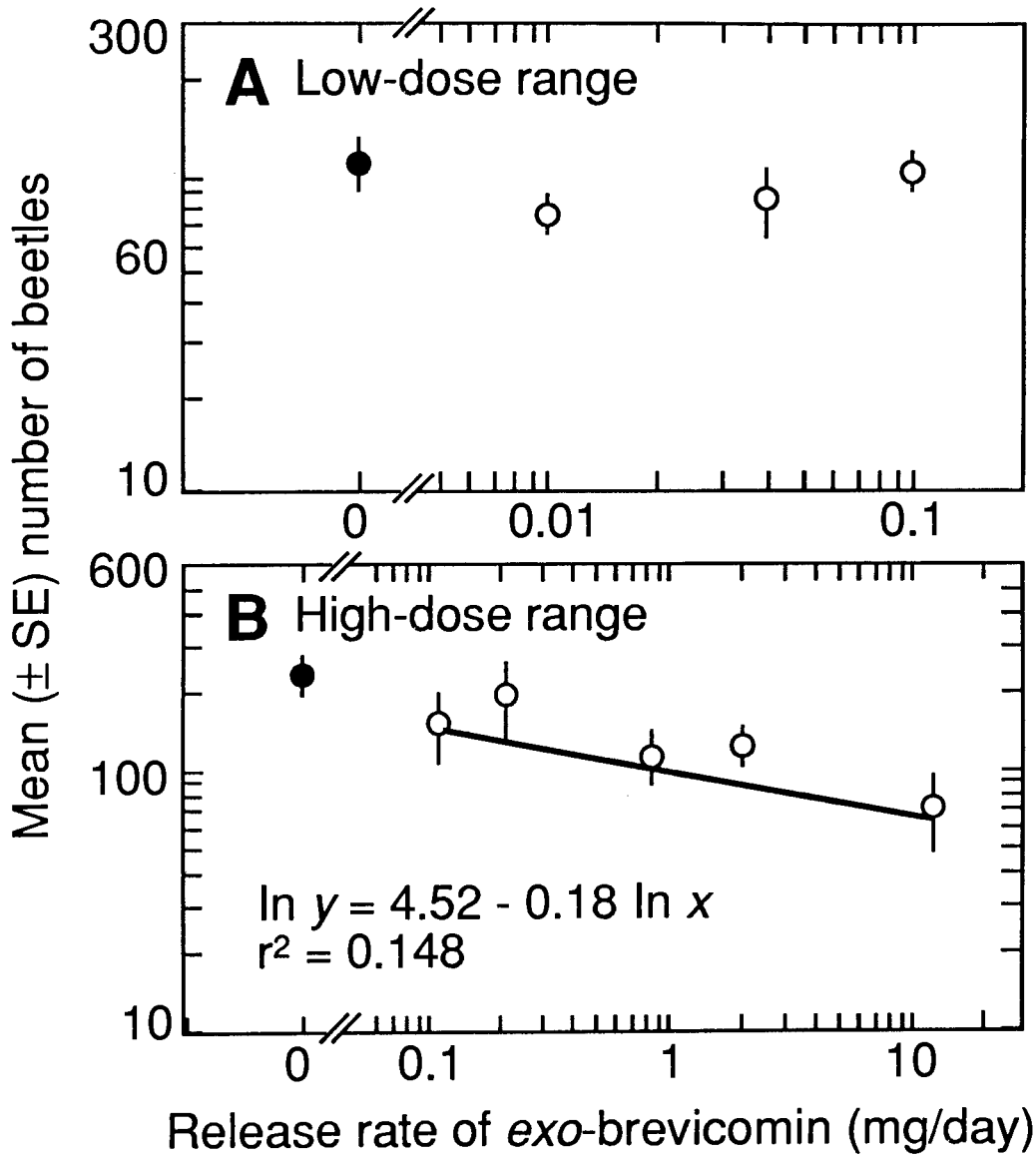


Figure 24. The effects of *exo*-brevicomin, released at various rates over a low (A) and high range (B), on the capture of *D. ponderosae* in multiple-funnel traps baited with myrcene and a 13:87 mix of *cis*- and *trans*-verbenol near Princeton BC. The slope of the regression line is significantly different from zero (t test, P=0.057).

mg/day (within the range I tested), *exo*-brevicomin caused attraction of *D. ponderosae* in similar stands of lodgepole pine near Princeton BC. The main difference between the two studies was trap spacing. Borden et al. (1987b) set traps at distances greater than 25 m while I set traps 10-15 m apart. Treatment effects may have prevented assessment of response to *exo*-brevicomin at low release rates. Inhibition of *D. ponderosae* to *exo*-brevicomin at high release rates is consistent with other studies (Rudinsky et al. 1974; Pitman et al. 1978; Ryker and Rudinsky 1982; Borden et al. 1987b), demonstrating the multifunctionality of *exo*-brevicomin.

The lack of a dose-dependent response by *I. latidens* may reflect the lack of large-aggregations situations for *I. latidens* occur. A dose-dependent response over a broad range of release rates should not be expected if that range does not occur in nature. The number of individuals involved in mass attacks by *I. latidens* tend to be low, relative to those of *D. ponderosae* and *I. pini* (personal observation). In contrast, *I. pini* can aggregate in large numbers (>2,000-3,000 galleries per log) (personal observation). The probability is high that *I. pini* individuals would have the opportunity to differentiate between ipsdienol released at very low release rates and ipsdienol released at very high rates.

The dose-dependent response by *I. pini* to its pheromone is sex-specific (Figs. 24A,B) and, therefore, probably not used to maintain reproductive isolation. Both sexes aggregate in response to the pheromone and both should benefit equally if the production of ipsdienol at high release rates indicated a higher probability of finding a conspecific. It is more probable that the sex-specific, dose-dependent response of *I. pini* reflects differential benefits to females and males as in other scolytid species (Byers 1989b).

In polygynous species of bark beetles, the production of pheromone by males signifies to females that there are galleries available for breeding. As long as pheromone is produced, females should have galleries to enter, particularly since several females can

join the same male (Kirkendall 1983). Pheromone production by males of four polygynous species, *I. paraconfusus*, *I. grandicollis*, *I. calligraphus* and *I. typographus*, seems to decrease as males acquire females, and apparently ceases as harems are filled (Borden 1967; Werner 1972b; Svihra 1982; Birgerrson et al. 1984; Schlyter and Löfqvist 1986; Birgerrson and Leufven 1988; Byers 1989b). The total production of pheromone emanating from a single host should increase as more males establish galleries and start producing pheromones. The response of females should be directly related to the production of pheromone, therefore, since more pheromone signifies more empty galleries with males.

Males, on the other hand, are searching for access to suitable breeding material in order to gain access to females. The production of large amounts of pheromone may indicate resource-rich host material but the benefits would not necessarily be conferred upon responding males. The number of potential sites is finite and pheromone produced at high rates would indicate that most of the sites are occupied. Dose-dependent behavior should be evident in females but not in males of polygynous species.

In contrast to *I. latidens* and *I.pini*, *Dendroctonus ponderosae* is a monogamous species where females initiate attack and are joined by males (Furniss and Carolin 1980). As in *I. pini*, however, large numbers of individuals are involved in mass attacks by *D. ponderosae* and the probability is high that individual *D. ponderosae* would have the opportunity to differentiate pheromone released at different rates. My data failed to show a dose-dependent response to a verbenol mix (Figs. 23A,B), due largely to contamination of verbenol lures with verbenone. It is probable that *D. ponderosae* does show non-sex-specific, dose-dependent responses to verbenols. *Dendroctonus ponderosae* attack standing, living trees and must achieve a threshold attack level in order to overcome the defenses of a tree (Berryman 1982; Berryman et al. 1985). Verbenols released at high rates would indicate host material that can be colonised and should benefit both males and females. A dose-dependent response by both sexes of *D.*

ponderosae to verbenols would facilitate host colonisation and help maintain reproductive isolation.

2.3.4 The effects of ethanol on the attraction of *Ips latidens* and *I. pini* to their respective pheromones

2.3.4.1 Introduction

Throughout my studies I have used two types of lures (C-flex® and bubble-cap) for releasing ipsenol and ipsdienol. Both lures utilised solutions of pheromones in order to minimise losses to oxidation and polymerisation. The C-flex® lures, containing ethanol solutions, were used in the earlier experiments while the later studies used bubble-cap lures containing solutions of 1,3-butanediol. The major advantage of the latter type of lure was the lack of release of 1,3-butanediol. In contrast the C-flex® lures released ethanol at a rate of approximately 5 mg/day at 24 °C (determined by weight loss), thereby confounding interpretation of results. Several species of Scolytidae, particularly ambrosia beetles, use ethanol as a kairomone (Borden 1982). Therefore it is possible that ethanol may affect the behavioral responses of *I. latidens* and *I. pini*.

My objective was to verify my assumption that ethanol is not a kairomone for either *I. latidens* or *I. pini*. I tested the two following hypotheses: 1) attraction of *I. latidens* would not be affected by ethanol nor by the interaction of ethanol with ipsenol; and 2) attraction of *I. pini* would not be affected by ethanol nor by the interaction of ethanol with ipsdienol.

2.3.4.2 Materials and Methods

PheroTech Inc. (Delta BC) supplied the following polyethylene, bubble-cap lures: 1) 1,3-butanediol (chemical purity, >99%); 2) ethanol (chemical purity, >99%);

3) (±)-ipsenol (chemical purity, 98%) in solution with 1,3-butanediol; and 4) (±)-ipsdienol (chemical purity, 98%) in solution with 1,3-butanediol. The release rates of ipsenol and ipsdienol were approximately 0.2-0.3 mg/day at 24 °C (determined by collection of volatiles on Porapak-Q) while that of ethanol was approximately 8 mg/day at 24 °C (determined by weight loss).

In all experiments, 12-unit, multiple-funnel traps (Lindgren 1983) (PheroTech Inc., Delta BC) were set in grids of 2 x 2 in mature stands of lodgepole pine near Princeton BC. Each trap was suspended by rope such that the top funnel of each trap was 1.5-1.8 m above ground. No trap was within 2 m of any tree. Treatments were randomly assigned within replicates. Sexes of captured *I. pini* were determined using declivital characters (Lanier and Cameron 1969) while those of captured *I. latidens* were determined by dissection and examination of genitalia.

In Experiment 1, I tested the effects of ethanol, and the interaction between ethanol and ipsenol, on the attraction of *I. latidens*. Seven replicate grids were set from 6 to 31 Aug, 1989. The treatments were as follows: 1) 1,3-butanediol control; 2) ethanol alone; 3) ipsenol alone; and 4) ipsenol with ethanol. The effects of ethanol, and the interaction between ethanol and ipsdienol, on the attraction of *I. pini* were tested in Experiment 2. Ten replicate grids were set from 25 July to 19 Aug, 1989. The treatments were as follows: 1) 1,3-butanediol control; 2) ethanol alone; 3) ipsdienol alone; and 4) ipsdienol with ethanol.

The data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). Trap catches were transformed by $\ln(Y+1)$, to remove heteroscedasticity, while sex ratio data were normalised by an arcsin transformation. Homoscedastic data were subjected to analysis of variance (ANOVA) using replicate, ethanol, ipsenol/ipsdienol, and the interaction between ethanol and ipsenol/ipsdienol, as model factors.

2.3.4.3 Results and Discussion

Ethanol had no effect on the responses of either *I. latidens* or *I. pini* (Figs. 25A,B). The interactions between ipsenol and ethanol, and ipsdienol and ethanol, had no significant effects on the responses of *I. latidens* and *I. pini*, respectively [ANOVA, F(1,26), P=0.349 and F(1,18), P=0.911]. The sex ratio of *I. latidens* caught in traps baited with ipsenol alone did not differ from that of *I. latidens* caught in traps baited with ipsenol and ethanol (t test, df=3.6, P=0.64). Similarly, the sex ratio of *I. pini* caught in traps baited with ipsdienol did not differ from that of *I. pini* caught in traps baited with ipsdienol and ethanol (t test, df=10.5, P=0.44). The mean ($\pm$SE) proportions of male *I. latidens* and *I. pini* responding to ipsenol and ipsdienol, respectively, were 0.08 $\pm$ 0.039 and 0.22 $\pm$ 0.021, respectively. The sex ratios of beetles caught in traps baited with the remaining treatments were not determined due to low numbers (all catches <10).

These data indicate that results of experiments that used C-flex® lures containing ethanol solutions of ipsenol or ipsdienol, in the absence of any other treatment(s), can be attributed solely to ipsenol or ipsdienol, respectively. In the presence of other treatments, however, interpretation of results must still consider the possible interaction of these other treatments either with ethanol or with the combinations of ethanol and ipsenol/ipsdienol.

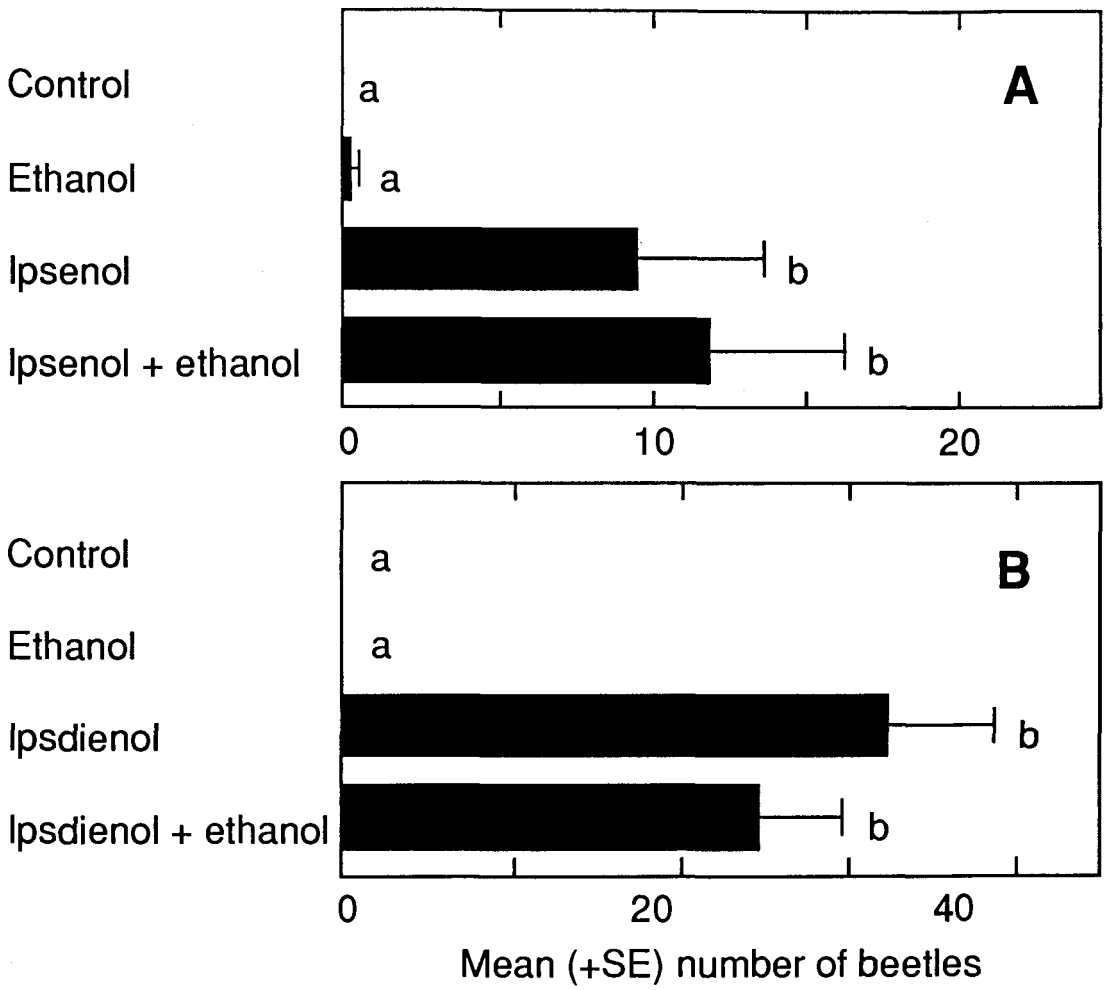


Figure 25. The effect of ethanol on the number of *I. latidens* (A) and *I. pini* (B) (B) captured in multiple-funnel traps near Princeton BC from 6 to 31 Aug, 1989 (n=7) and from 25 July to 19 Aug, 1989 (n=10), respectively. Mean trap catches, for the same species, followed by the same letter are not significantly different at P=0.05 [Duncan's Multiple Range test on data transformed by ln(Y+1)].

2.4 ISOLATION BY SYNOMONES

Synomones (= mutualistic allomones) are one of three classes of semiochemicals that convey information between heterospecifics (Bethe 1932; Brown 1968; Brown et al. 1970; Otte 1974; Nordlund and Lewis 1976). Natural selection has favored: 1) individuals that produced synomones that affected the behavior of heterospecifics; and 2) individuals that modified their behavior in response to synomones produced by heterospecifics (Otte 1974; Nordlund and Lewis 1976; Nordlund 1981; Dicke and Sabelis 1988; Whitman 1988). Synomones are similar to pheromones in that both are used to convey signals or messages (i.e. to communicate) between individuals; a function that should be beneficial for both participants (Burghardt 1970; Law and Regnier 1971; Otte 1974; Nordlund and Lewis 1976; Rutowski 1981). Synomones can be defined as semiochemicals used in interspecific communication.

The major difference between synomones and the two other classes of transspecific semiochemicals, kairomones and allomones(= antagonistic allomones), is that the latter do not function as means of communication. Kairomones should benefit the responding individuals by providing contextual information about the environment (Brown et al. 1970; Whittaker and Feeny 1971; Nordlund and Lewis 1976). In contrast, allomones should benefit individuals that produce them due to predictable responses on the part of the receiver that are probably detrimental to the fitness of the receiver, such as in crypsis or aggressive mimicry (Brown et al. 1970; Nordlund and Lewis 1976; Nordlund 1981; Dicke and Sabelis 1988; Whitman 1988). The potential benefits from the use of kairomones and allomones are apt to be unilateral; only one of the participants in the transfer of information probably acquires a higher fitness. In contrast, the potential benefits from the use of synomones (and pheromones) are apt to be bilateral; both participants should benefit from the transfer of information. Therefore, we should expect that the use of synomones in a community of bark beetles should be

inherently stable; more so than the use of kairomones or allomones which should be inherently unstable.

The relative importance of synomones in community structure must be dependent on the benefits to individuals that engage in interspecific communication. For bark beetles, the benefits probably relate to both functions of semiochemicals: 1) to locate mates; and 2) to locate breeding material for offspring. With respect to mate location, signals that communicate the presence of heterospecifics within or adjacent to aggregations of conspecifics should convey information concerning the relative probability of finding a conspecific for the purpose of mating (Cardé 1986). Similarly, individuals that signal to heterospecifics minimise the possibility of heterospecific matings. Synomones can function to ensure reproductive isolation.

Synomones can also function with respect to the breeding resource. Bark beetles show considerable species-specificity in their choice of host with regard to species, age, and part of a tree or log (Furniss and Carolin 1980; Stark 1982). The competitive exclusion principle (Hardin 1960) suggests that these choices are a consequence of differential abilities in different host types. Individuals should use synomones in attempts to minimise interspecific interactions amongst their offspring (Nordlund 1981). In bark beetles, therefore, it is quite probable that synomones are as important as pheromones in determining community structure with respect to the use of semiochemicals.

2.4.1 Dose-dependent responses of *Ips latidens*, *I. pini* and *Dendroctonus ponderosae* to their respective synomones

2.4.1.1 Introduction

My preliminary experiments disclosed that *Ips latidens*, *I. pini* and *Dendroctonus ponderosae* are inhibited in their attraction to their respective pheromones by the

pheromones of heterospecifics. Specifically, ipsdienol reduces the attraction of *I. latidens* and *D. ponderosae* to their respective pheromones (unpublished results) and a 13:87 mixture of *cis*- and *trans*-verbenol inhibits the attraction of *I. pini* to its pheromone (unpublished results). The function of these semiochemicals in an interspecific context can facilitate reproductive isolation and minimise interspecific competition. These semiochemicals, therefore, can act as synomones.

My objectives were threefold. Firstly, I planned to verify that ipsdienol is a synomone between *I. pini* and *I.latidens* and between *I. pini* and *D. ponderosae*, and that verbenols are synomones between *D. ponderosae* and *I. pini*. Secondly, I planned to determine if ipsenol and *exo*-brevicomin act as synomones. And lastly, I planned to determine if and how synomones released at different rates affect the response of beetles. A dose-dependent response, over a broad range of release rates, should be expected for individuals of species that breed in adjacent areas, reflecting the relative probabilities of mating opportunities and levels of interspecific competition. Alternatively, an all-or-none response should be expected for individuals that do not breed in close proximity to each other. I tested the eight following hypotheses: 1-3) the attraction of *I. latidens* to (±)-ipsenol would be inhibited by (±)-ipsdienol, *exo*-brevicomin or a mixture of *cis*- and *trans*-verbenol, respectively, in a dose-dependent fashion; 4-6) the attraction of *I. pini* to (±)-ipsdienol would be inhibited by (±)-ipsenol, *exo*-brevicomin or a mixture of *cis*- and *trans*-verbenol, respectively, in a dose-dependent fashion; and 7-8) the attraction of *D. ponderosae* to the combination of *exo*-brevicomin, *cis*- and *trans*-verbenol would be inhibited by (±)-ipsenol or (±)-ipsdienol, respectively, in a dose-dependent fashion.

2.4.1.2 Materials and Methods

(-)-β-Phellandrene was obtained from H.D. Pierce, Jr. (Dept. of Chemistry, Simon Fraser University). Phero Tech Inc. (Delta BC) supplied the following chemicals and lures: 1-5) β-myrcene, (±)-ipsenol, (±)-ipsdienol, (±)-*exo*-brevicomin and a 13:87 mixture of *cis*- and *trans*-verbenol (chemical purities, all>98%); 6) (±)-*exo*-brevicomin (chemical purity, 98%) capillary-tube lures; 7) bubble-cap lures containing a solution of (±)-ipsenol (chemical purity, 98%) in 3-butanediol; and 8) polyethylene, bubble-cap lures containing a 13:87 mixture of *cis*- and *trans*-verbenol (chemical purities, both 98%). The chiral ratios of both *cis*- and *trans*-verbenol were 83%(-):17%(+). The release rates of *exo*-brevicomin, ipsenol and the verbenols from these lures were approximately 0.21, 0.25 and 2.06 mg/day at 24 °C (determined by collection of volatiles on Porapak-Q).

β-Phellandrene was released from closed, polyethylene, micro-centrifuge tubes (1.8 mL) (Evergreen Scientific, Los Angeles CA) at a rate of approximately 8 mg/day at 27-30 °C (determined by weight loss). Myrcene was released from closed, polyethylene, screw-cap bottles (15 mL) (Ampak Inc., Richmond BC) at a rate of approximately 281 mg/day at 24 °C (determined by weight loss).

Ipsenol (except in Experiment 1) and ipsdienol were released from 10- to 100-cm-lengths of C-flex® tubing (ID=1.6 mm; OD=3.2 mm) (Concept Inc., Clearwater FL), filled with ethanol solutions of either ipsenol or ipsdienol and heat-pressure sealed at both ends. In Experiment 1, ipsenol was released from bubble-cap lures.

In Experiment 8, *cis*- and *trans*-verbenol were released together from the following devices: 1-2) open, polypropylene, micro-centrifuge tubes (1.5 mL) (Quality Scientific Plastics, Petaluma CA), containing either one or five 2-cm-long, glass, capillary tubes (ID=1.5 mm; OD=1.8 mm), each sealed at one end and filled with verbenols; 3) closed, polyethylene, micro-centrifuge tubes (250 μL) (Evergreen Scientific, Los Angeles CA); and 4-5) either one or three bubble-cap lures. In Experiment 4, the third device

was replaced by three open, polypropylene, micro-centrifuge tubes (1.5 mL) (Quality Scientific Plastics, Petaluma CA), each containing five 2-cm-long, glass, capillary tubes (ID=1.5 mm; OD=1.8 mm), sealed at one end and filled with verbenols. In Experiments 9 and 10, verbenols were released from bubble-cap lures.

In Experiment 7, *exo*-brevicomin was released from the following devices: 1) open, polypropylene, micro-centrifuge tubes (1.5 mL) (Quality Scientific Plastics, Petaluma CA), each containing one Microcap® disposable pipette (2 μL) (Drummond Scientific Co., Broomall PA), sealed at one end and filled with *exo*-brevicomin; 2) closed, polyethylene, micro-centrifuge tubes (250 μL) (Evergreen Scientific, Los Angeles CA); 3-5) open, polypropylene, micro-centrifuge tubes (1.5 mL) (Quality Scientific Plastics, Petaluma CA) containing either one, three or ten 2-cm-long, glass, capillary tubes (ID=1.5 mm; OD=1.8 mm), each sealed at one end and filled with *exo*-brevicomin. In Experiment 3, the second device was replaced by open, polypropylene, micro-centrifuge tubes (1.5 mL) (Quality Scientific Plastics, Petaluma CA) containing three Microcap® disposable pipettes (2 μL) (Drummond Scientific Co., Broomall PA), sealed at one end and filled with *exo*-brevicomin. In Experiments 9 and 10, *exo*-brevicomin was released from capillary-tube lures.

In all experiments, grids of 8-unit, multiple-funnel traps (Lindgren 1983) (Phero Tech Inc., Delta BC) were set in mature stands of lodgepole pine. Replicate grids were placed at least 100 m apart, and traps were spaced 10-15 m apart within each replicate. Each trap was suspended between trees such that the top funnel of each trap was 1.3-1.5 m above ground. No trap was within 2 m of any tree. Experiments 1, 9 and 10 were conducted near Princeton BC to exploit high population levels of *I. latidens* and *D. ponderosae*. Experiments 5 and 6 were conducted near Williams Lake BC, while Experiments 7 and 8 were conducted near Princeton BC, to exploit high population levels of *I. pini*. Experiments 2-4 were conducted near Williams Lake in anticipation of high population levels of *I. latidens* which failed to materialise.

Experiments 1-4 determined the effects of different release rates of ipsdienol (low and high ranges), *exo*-brevicomin and a 13:87 mixture of *cis*- and *trans*-verbenol, respectively, on the attraction of *I. latidens* to ipsenol. Five replicate grids (2 x 3) per experiment were set for Experiments 1 and 2 during the periods of 17 June to 20 July, 1989, and 27 June to 17 July, 1988, respectively, while four replicate grids (2 x 3) per experiment were set for Experiments 3 and 4 during the period of 15 June to 17 July, 1988. The treatments for Experiment 1, randomly assigned within each replicate, were as follows: 1) ipsenol; and 2-6) ipsenol with five ipsdienol treatments, differing only in release rates (Table 9). The treatments for Experiments 2-4 were similar to those for Experiment 1 except that β-phellandrene was added to all traps and ipsdienol was replaced by *exo*-brevicomin and the verbenol mix for Experiments 3 and 4 (Table 9). Experiments 5-8 determined the effects of different release rates of ipsenol (low and high ranges), *exo*-brevicomin and the verbenol mixture, respectively, on the attraction of *I. pini* to ipsdienol. Four replicate grids (2 x 3) were set for Experiment 5 during the period of 10 to 17 Aug, 1988. Five replicate grids (2 x 3) per experiment were set for Experiments 6-8 during the periods of 18 July to 9 Aug, 1988, 20 Aug to 29 Sept, 1987, and 20 Aug to 28 Sept, 1987, respectively. The treatments for Experiments 5 and 6, randomly assigned within each replicate, were as follows: 1) ipsdienol; and 2-6) ipsdienol with five ipsenol treatments, differing only in release rates (Table 9). The treatments for Experiments 7 and 8 were similar to those for Experiments 5 and 6 except that ipsenol was replaced by *exo*-brevicomin and the verbenol mixture, respectively (Table 9).

Experiments 9 and 10 determined the effects of different release rates of ipsenol and ipsdienol, respectively, on the attraction of *D. ponderosae* to the combination of myrcene, *exo*-brevicomin and the verbenol mixture. Five replicate grids (2 x 3) per experiment were set for Experiments 9 and 10 during the periods of 27 July to 4 Aug,

Table 9. Approximate release rates (μg/day at 24 °C, unless otherwise noted) of pheromones and synomones used in Experiments 1-10, conducted in stands of lodgepole pine in British Columbia in 1987-1989.

		Treatment					
	Experiment	Control	1	2	3	4	5
Ipsdienol	1 [a]	0	60	20	60	100	300
Ipsdienol	2 [b]	0	60	180	600	1,800	6,000
exo-Brevicomin	3 [b]	0	121	228	684	2,583	7,749
Verbenol mix	4 [b]	0	111	333	703	1,387	4,645
Ipsenol	5 [c]	0	0.06	0.6	6	60	600
Ipsenol	6 [c]	0	60	200	600	2,000	6,000
exo-Brevicomin	7 [c]	0	110	703	1387	2,056	4,645
Verbenol mix	8 [c]	0	121	206	228	2,583	7,749
Ipsenol	9 [d]	0	6	60	600	2,400	6,000
Ipsdienol	10 [d]	0	6	60	600	2,400	6,000

[a] With ipsenol released at approximately 0.2-0.3 mg/day.

[b] With ipsenol and β-phellandrene released at approximately 0.6 and 8 mg/day, respectively.

[c] With ipsdienol released at approximately 0.6 mg/day.

[d] With *exo*-brevicomin, verbenol mix and myrcene released at approximately 0.21. 2.06 and 281 mg/day, respectively.

1988, and 4 to 14 Aug, 1988, respectively. The treatments were as follows: 1) the combination of myrcene, *exo*-brevicomin and the verbenol mixture; and 2-6) myrcene, *exo*-brevicomin and the verbenol mixture with five ipsenol or ipsdienol treatments, respectively, differing only in release rates (Table 9).

In Experiments 1-4, 5-8, and 9 and 10, subsamples (n=30-50) of captured *I. latidens*, *I. pini* and *D. ponderosae*, respectively, for determination of sexes, were taken at random from catches to the lowest, medial and highest release rates of semiochemicals for each experiment. Sexes of captured *I. pini* were determined using declivital characters (Lanier and Cameron 1969) while those of captured *I. latidens* and *D. ponderosae* were determined by dissection and examination of genitalia.

Trap catch data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). Trap catch data were transformed by ln(Y+1) in Experiments 1, 2, 6 and 10, and ln(Y) in the remaining experiments, to remove heteroscedasticity. Homoscedastic data were subjected to two-way analysis of variance (ANOVA) using replicate and treatment as model factors. Five orthogonal contrasts were performed in each experiment, comparing the control against each semiochemical treatment. For each experiment, trap catch data, transformed by ln(Y), were regressed against the release rate of semiochemical, transformed by ln(X), using treatment as the only factor in a general linear model. Sex ratio data were analysed by Chi^2 tests of independence/association using the Minitab statistical package ver. 5.1.1 (Dept. Statistics, Pennsylvania State University, University Park PA).

2.4.1.3 Results

Regression analyses and orthogonal contrasts verified that most of the pheromones of *I. latidens*, *I. pini* and *D. ponderosae* are used as synomones as well. Ipsdienol inhibited the attraction of *I. latidens* to its pheromone, ipsenol, in a dose-dependent fashion over a

low range of release rates of ipsdienol (Fig. 26A), with significantly lower catches in all traps baited with ipsdienol than in controls (orthogonal contrasts, ANOVA, all $P<0.001$). Ipsdienol (over a higher range of release rates) totally inhibited the attraction of *I. latidens* to the combination of ipsenol and β-phellandrene at the four, highest release rates (orthogonal contrasts, ANOVA, all $P<0.001$) (Fig. 26B).

exo-Brevicomin and the verbenol mixture inhibited the attraction of *I. latidens* to the combination of ipsenol and β-phellandrene in dose-dependent fashions over broad ranges of release rates (Fig. 27A,B). Significantly fewer *I. latidens* were caught in traps baited with *exo*-brevicomin released at the fourth- and fifth-highest rates than in controls (orthogonal contrasts, ANOVA, $P=0.019$ and $P=0.046$, respectively). Significantly fewer *I. latidens* were caught in traps baited with the verbenol mixture than in controls (orthogonal contrasts, ANOVA, all $P<0.025$).

Similarly, dose-dependent inhibition of attraction of *I. pini* to its pheromone, ipsdienol, was caused by ipsenol, *exo*-brevicomin and the verbenol mixture (Figs. 28A,B;29A,B). Relative to controls, significantly fewer *I. pini* were caught in traps baited with ipsenol released at the highest rate in the low-range experiment (Fig. 28A) (orthogonal contrast, ANOVA, $P<0.001$) and in all traps baited with ipsenol in the high-range experiment (Fig. 28B) (orthogonal contrasts, ANOVA, all $P<0.033$). Significantly fewer *I. pini* were caught in traps baited with *exo*-brevicomin released at the third-, fourth- and fifth-highest rates relative to controls (orthogonal contrasts, ANOVA, $P=0.035$, $P=0.006$ and $P=0.047$, respectively). Significantly fewer beetles were caught in traps baited with the verbenol mixture released at the fourth- and fifth-highest rates (orthogonal contrasts, ANOVA, $P=0.002$ and $P<0.001$, respectively).

Dose-dependent inhibition of attraction of *D. ponderosae* to the combination of myrcene, *exo*-brevicomin and the verbenol mixture was caused by ipsdienol but not ipsenol (Figs. 30A,B). There were no significant differences in catches of *D. ponderosae* in traps baited with ipsenol relative to controls (orthogonal contrasts, all $P>0.200$).

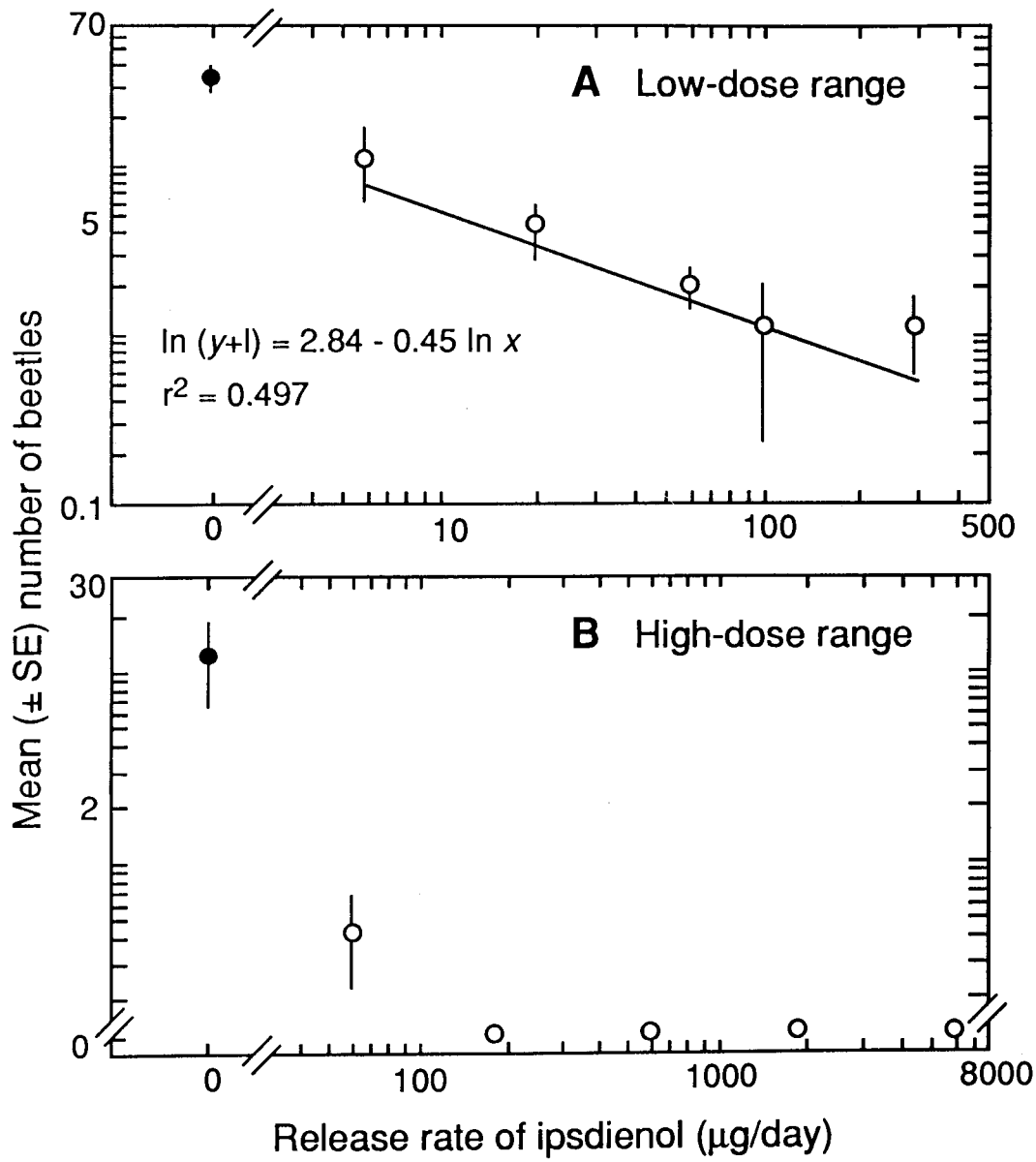


Figure 26. The effect of ipsdienol, released at various rates, over low (A) and high ranges (B), on the attraction of *I. latidens* to multiple-funnel traps baited with ipsenol (A) or the combination of ipsenol and β-phellandrene (B), in 1989 and 1988, respectively. The slope of the regression line is significantly different from zero (t test, $P<0.001$).

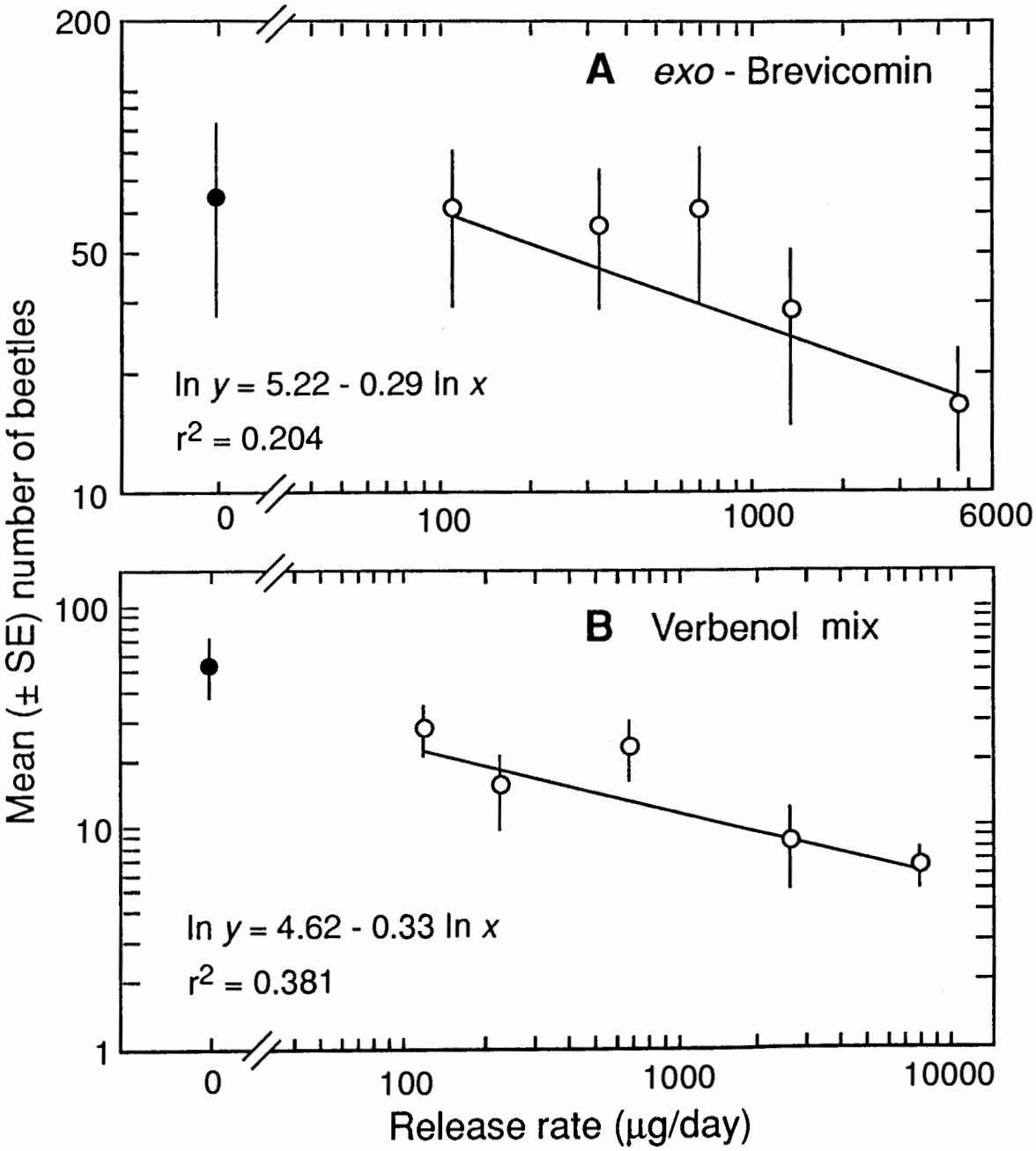


Figure 27. The effects of *exo*-brevicomin (A) and a 13:87 mix of *cis*- and *trans*-verbenol (B), released at various rates, on the attraction of *I. latidens* to multiple-funnel traps baited with the combination of ipsenol and β-phellandrene near Williams Lake BC in 1988. The slopes of the regression lines are significantly different from zero (t test, P=0.052 and P=0.004, respectively).

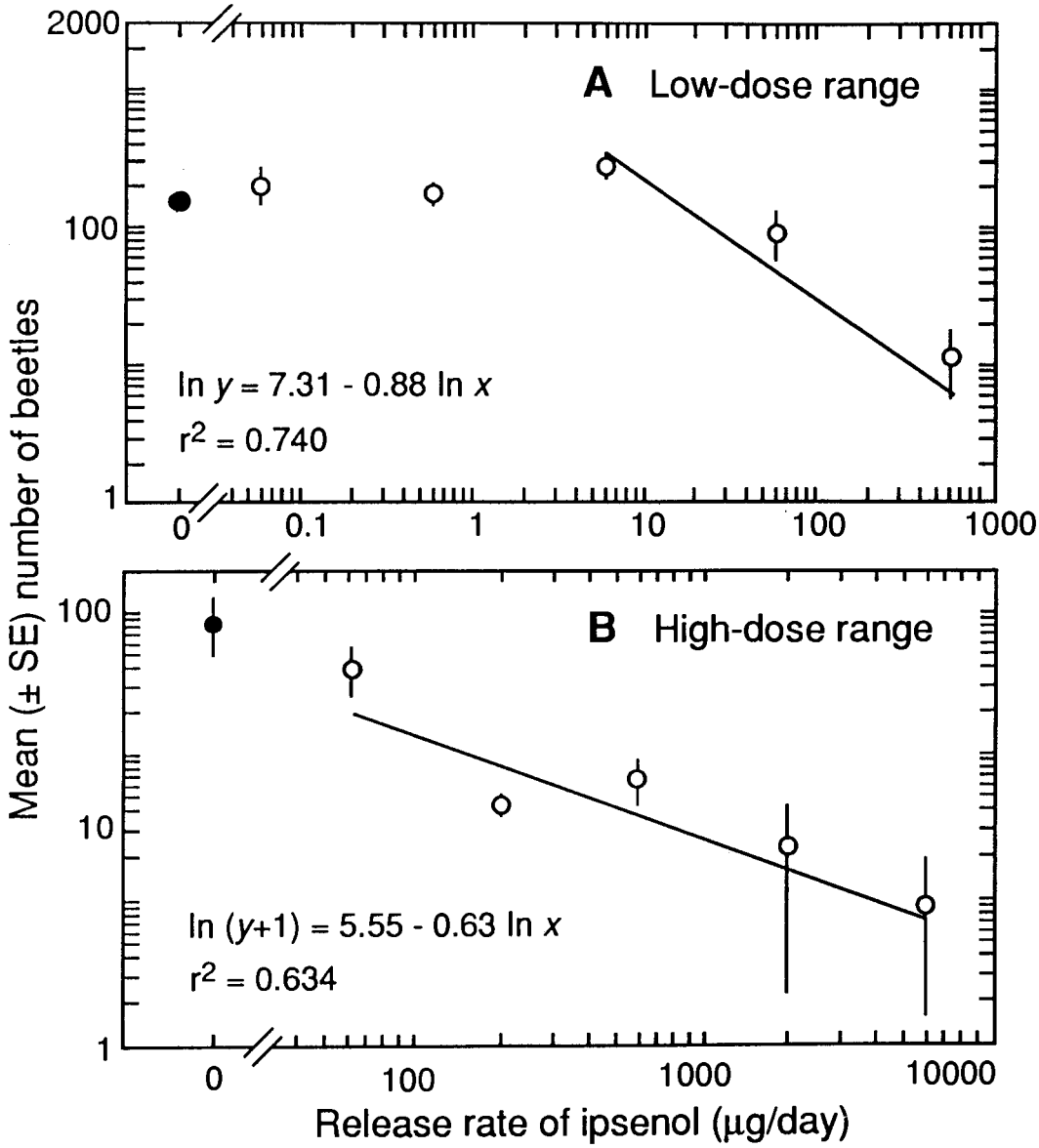


Figure 28. The effect of ipsenol, released at various rates, over low (A) and high ranges (B), on the attraction of *I. pini* to multiple-funnel traps baited with ipsdienol near Williams Lake BC in 1988. The slopes of the regression lines are significantly different from zero (t test, $P<0.001$ and $P<0.001$, respectively).

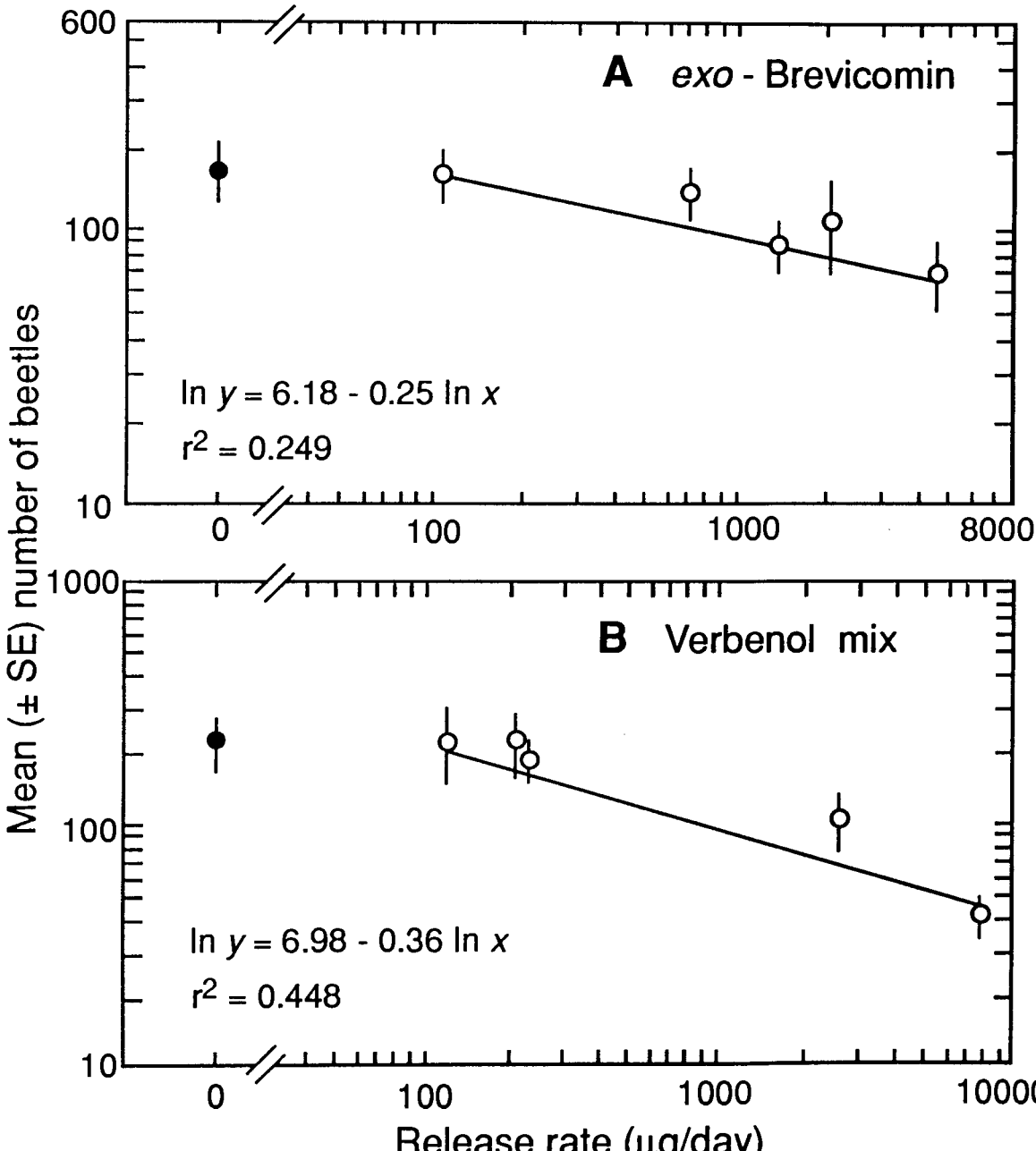


Figure 29. The effects of *exo*-brevicomin (A) and a 13:87 mix of *cis*- and *trans*-verbenol (B), released at various rates, on the attraction of *I. pini* to multiple-funnel traps baited with ipsdienol near Princeton BC in 1987. The slopes of the regression lines are significantly different from zero (t test, $P=0.011$ and $P<0.001$, respectively).

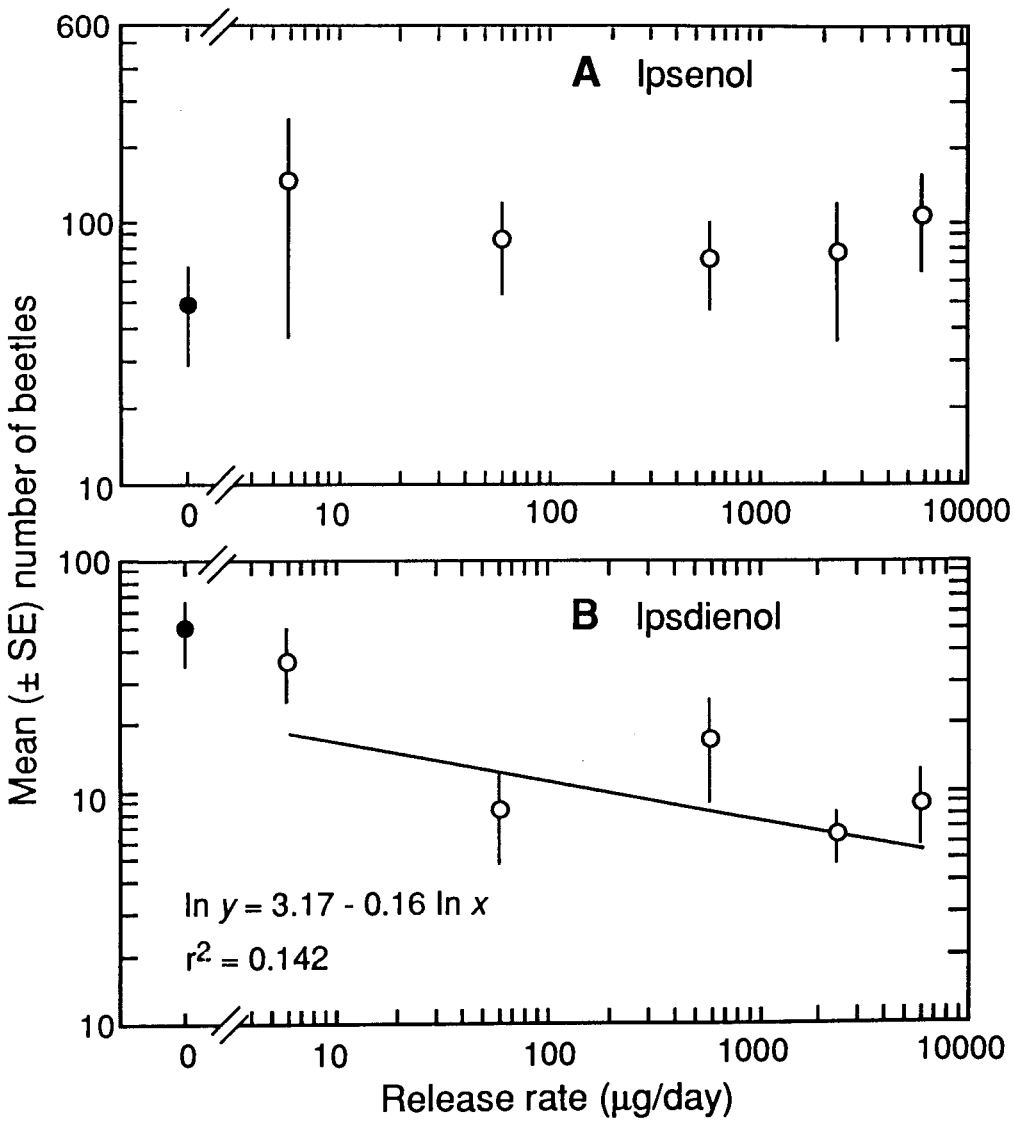


Figure 30. The effects of ipsenol (A) and ipsdienol (B), released at various rates, on the attraction of *D. ponderosae* to multiple-funnel traps, baited with the combination of myrcene, *exo*-brevicomin, *cis*- and *trans*-verbenol, near Princeton BC in 1988. The slope of the regression line is significantly different from zero (t test, P=0.064).

Significantly fewer beetles were caught in traps baited with ipsdienol released at the four highest rates (orthogonal contrasts, ANOVA, all $P<0.01$).

There were no significant changes in sex ratios in Experiments 1 and 3-10 (Chi^2 test, all df=2, all $P>0.05$). Effects of treatment on sex ratio were not determined in Experiment 2 due to insufficient numbers of *I. pini* caught in most of the treatments.

2.4.1.4 Discussion

My results demonstrate that ipsenol, ipsdienol, *exo*-brevicomin and a 13:87 mixture of *cis*- and *trans*-verbenol act as pheromones and synomones for *I. latidens*, *I. pini* and *D. ponderosae*. These semiochemicals act as synomones by generally inhibiting the attraction of heterospecifics to their respective pheromones (Figs. 26A,B-29A,B;30B), a response typical of many interactions among bark beetles. Interspecific inhibition of response to pheromones occurs among various pairs of bark beetle species in western North America such as between *Ips paraconfusus* and *I. pini* (Birch and Wood 1975; Birch and Light 1977; Birch et al. 1977; Light and Birch 1977; Birch 1978; Birch et al. 1980a), and between *Dendroctonus ponderosae* and *I. paraconfusus* (Byers and Wood 1980, 1981). In Europe, reproductive isolation among six *Ips* species seems to be facilitated by species-specificity in pheromone composition and mutual inhibition of responses (Kohnle et al. 1986, 1988). In the southern United States, four sympatric species of bark beetles breed in loblolly pine, often with three or all four present in the same tree (Birch 1978; Dixon and Payne 1979; Birch and Svihra 1979; Svihra et al. 1980; Paine et al. 1981). Partitioning of the host seems to occur primarily through partitioning of the use of pheromones and mutual inhibition (Vité and Francke 1976; Birch 1978; Vité et al. 1978; Birch et al. 1980b; Svihra et al. 1980; Byers 1989a,b).

The only exception, among *I. latidens*, *I.pini* and *D. ponderosae*, to the phenomenon of mutual inhibition was the lack of any effect of ipsenol on the attraction

of *D. ponderosae* to its own pheromone. It is possible that *D. ponderosae* do not have antennal receptor cells keyed to ipsenol, a pheromone commonly used by species of *Ips* (Borden 1982). However, a congeneric species, *D. terebrans*, does have antennal receptor cells keyed to ipsenol (Delorme and Payne 1990). Ipsenol has not been tested on antennae of any other species of *Dendroctonus*.

Alternatively, the lack of inhibition by *D. ponderosae* to ipsenol may reflect a lack of situations when ipsenol is released at the same time as the pheromones of *D. ponderosae*. If such situations do not occur in nature then there is no opportunity for selection to favor one behavior over another. *Ips latidens* may attack areas unsuccessfully attacked by *D. ponderosae* in previous years, or in areas adjacent to current galleries of *D. ponderosae* but only after production of one or more of the pheromones of *D. ponderosae* has ceased.

A third possibility in the lack of inhibition may be that the benefits in finding an ephemeral host may offset any risk of interspecific mating and/or competition, especially if the latter can be resolved at a later time. *Dendroctonus ponderosae* may wait till they sample the resource before leaving. It is not clear, however, why this would occur in the interaction between *D. ponderosae* and *I. latidens* but not in any of the other interspecific pairings.

My results revealed two characteristics of the inhibitory responses of the three species to synomones that have not previously been demonstrated with other species of bark beetles. Firstly, there is some variation in the treshhold level of release rate of a synomone that is required to initiate inhibition. The attraction of *I. latidens* to its pheromone was inhibited by ipsdienol released at the lowest rate (6 μg/day at 24 °C) (Fig. 26A) while the attraction of *I. pini* to ipsdienol did not begin to decrease until the release rate of verbenol exceeded 200 μg/day at 24 °C (Fig. 29B). The variation in threshold levels may reflect variation in the competitive ability of heterospecifics or the critical need for reproductive isolation in some species. Individuals of species such as *I.*

latidens may not be able to afford the risks involved in not maintaining reproductive isolation due to parameters such as fat reserves, while individuals of species such as *I. pini* and *D. ponderosae* may have a longer life expectancy before finding suitable host material.

The second feature of the inhibitory response concerns the phenomenon of dose-dependent responses over a large range of values. The variation in response may reflect variation in the guidance system and the cost associated with such variation. We can expect that the relative proportions of the pheromones of two species that breed in adjacent areas along the trunk of a tree should vary across the area of overlap of airspace, even if the areas on the host are clearly separate. An individual can always walk or fly to the appropriate location after the initial landing. Alternatively, the dose-dependent behavior may reflect differential benefits to individuals. Given the ephemeral and patchy nature of host material, it is possible that some individuals of a species may accept the consequence of interspecific competition or lack of mating opportunities if the likelihood of finding another suitable host is low. The probability of landing at a site should be related to the relative release rates of the two pheromones, which in turn should be related to their relative fitnesses. The relative fitness of an individual should be a consequence of the likelihood of that individual finding a better host, which in turn is dependent on various factors such as host density, epidemiology of the bark beetle infestation and the fat reserves in that particular individual relative to the average individual in the population. Fat reserves provide individuals with an opportunity to assess part of the likelihood of finding a better host.

The lack of a dose-dependent response over a broad range, such as that shown by *I. latidens* to the pheromone of *I. pini* (Fig. 26B), suggests that these species seldom breed in adjacent areas and are probably quite disparate in their host requirements. The effects of such interactions should greatly facilitate the maintenance of reproductive isolation as well as minimise interspecific competition among *I. latidens*, *I. pini* and *D.*

ponderosae, and between these and other species of bark beetles that may use combinations of ipsenol, ipsdienol, *exo*-brevicomin, and *cis*- and *trans*-verbenol as pheromones. In cases where dose dependence does occur, some other proximal factor is required to maintain absolute reproductive isolation.

3 GEOGRAPHIC VARIATION IN THE USE OF A PHEROMONE BY ONE SPECIES OF BARK BEETLE

In the preceding chapters, I described an example of one major type of community pattern: species-specificity in the use of semiochemicals among three sympatric species of bark beetles. A second major type of pattern, associated with communities, is the variation of the pattern of species-specificity between geographically-separate communities (Roelofs 1980; Cardé and Baker 1984; Cardé 1986). If species-specificity in a community is a consequence of the selection pressures involved in the maintenance of reproductive and ecological isolation then the pattern of geographic variation of species-specificity should be related to variation in competition pressures. Geographic variation should be dependent on the relative number of species, and their relative competitive abilities, within different communities. The acuity of the communication system should be greater in areas that are species rich relative to areas that are species poor. As well, if one pheromone has physiochemical properties that make it a superior transmission channel then the use of that pheromone should fall to individuals of the most-competitive species. In areas where that species is absent, the use of the same pheromone should fall to individuals of the next most-competitive species.

3.1 *IPS PINI:* PRODUCTION AND RESPONSE TRAITS

Every communication system consists of two participants: 1) a producer of a signal; and 2) a receiver of a signal (Burghardt 1970). The evolution of communication systems requires parallel changes on both sides of the system (Löfstedt et al. 1989). Genetic coupling of traits pertaining to the participants (sender and receiver) could facilitate such evolution (Cardé 1986; Hansson et al. 1987). However, it is more probable that such traits are not necessarily linked genetically and that their coexistence in a population is a

consequence of coevolution (Löfstedt et al. 1989; Löfstedt 1990). In the European corn borer, *Ostrinia nubilalis*, the genes controlling pheromone production by females are not linked genetically to the genes controlling perception by males (Roelofs et al. 1987; Baker 1989; Löfstedt et al. 1989). Variation in the production of a signal, therefore, may not necessarily be associated genetically with a similar variation in the response to a signal. Similarly the lack of variation in one trait should not imply a lack of variation in the second trait.

I planned to study pheromone production and response traits separately in various populations of *I. pini*, a scolytid species that is transcontinental in distribution and breeds in various species of conifers, particularly pines and spruces (Furniss and Carolin 1980; S.L. Wood 1982). Like many scolytids, *I. pini* aggregates rapidly and in large numbers to suitable hosts (Anderson 1948) in response to the male-produced pheromone, ipsdienol (Vité et al. 1972; Stewart 1975). Geographic variation in the use of ipsdienol as a pheromone is known (Lanier 1972; Birch 1978). Ipsdienol exists as two optical isomers or enantiomers (Fig. 31), differing only in the absolute configuration around the chiral center. Males from California (Stewart 1975; Birch et al. 1980a) and Idaho (Plummer et al. 1976) produce primarily (-)-ipsdienol while beetles from New York produce a 65:35 mixture of (+) and (-) enantiomers (Lanier et al. 1980). California beetles are attracted by (-)-ipsdienol but are repelled by (+)-ipsdienol (Birch et al. 1980a), while New York beetles respond best to a racemic mixture (equal quantities of (+)- and (-)-ipsdienol) (Lanier et al. 1980).

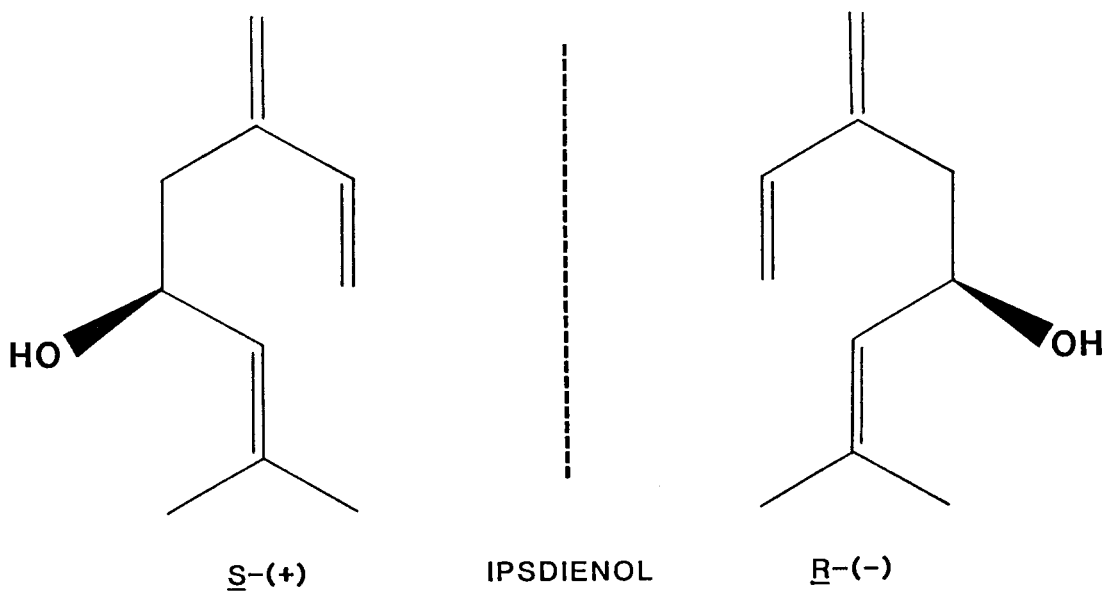


Figure 31. Enantiomers of the aggregation pheromone, ipsdienol, produced by male *I. pini*.

3.1.1 Inter- and intrapopulation variation in the production of chiral ipsdienol by male *Ips pini*

3.1.1.1 Objective and Hypotheses

My objective was to describe the interpopulation variation in the production of the pheromone, ipsdienol, by *I. pini*. To date, variation within populations of *I. pini* has been masked by the need for pooled samples of 300 or more beetles to obtain a sufficient quantity of ipsdienol for a single determination of chirality. It is now possible to determine the chirality of as little as 25 ng of ipsdienol by splitless, capillary, gas chromatography following derivatisation to acetyl lactate diastereomers (Slessor et al. 1985). I tested the two following hypotheses: 1) the mean quantity and chirality of ipsdienol produced by male *I. pini* would differ between populations; and 2) the coefficients of variation for quantity and chirality would also exhibit interpopulation variation.

3.1.1.2 Materials and Methods

Populations of *I. pini* were collected from eight localities in North America. I collected bolts of lodgepole pine infested with live broods of *I. pini* from Kimberley and Princeton BC in 1984, Osprey Lake, Pemberton and Williams Lake BC in 1986, and Radium BC in 1987. Infested bolts were placed in rearing cages in the laboratory and adult beetles were collected after emergence as mature adults. In 1984 and 1985 newly-emerged adults were transported by aircraft from Newcomb NY and Hat Creek CA. Red pine was the brood host for beetles from Newcomb; lodgepole pine was the brood host for beetles from the other seven localities.

Using the gelatin-pill-capsule technique (Borden 1967), adult males from each of the eight populations were restrained on uninfested bolts of lodgepole pine collected near

Princeton BC. They were allowed to bore into the bark and feed for 24-48 hrs. Abdomens from individual males were removed and each was crushed in 150 μL of pentane containing (±)-3-octanol (4.1 ng/μL) as an internal standard. These extracts were analysed by splitless, capillary, gas chromatography (Hewlett Packard HP 5890 using a 30 m x 0.25 mm ID fused silica column), before and after derivatisation to acetyl lactate diastereomers (Slessor et al. 1985). Retention times of ipsdienol and its derivatives were determined with (±)-ipsdienol obtained from Borregaard A.S. (Sarpsborg, Norway) and chiral assignments were made according to Slessor et al. (1985). The identities and integrities of ipsdienol acetyl lactate diastereomers were verified by mass spectrometry using splitless, capillary, gas chromatography (Hewlett Packard HP 5985B).

Data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). Quantities of ipsdienol were transformed by ln(Y+1) and subjected to one-way analysis of variance (ANOVA). Scheffe's Multiple Comparison test was performed when $P<0.05$. Chirality data was not subjected to ANOVA due to severe deviations from normality (bimodality in some data sets). For each population, coefficients of variation (CV) were determined for quantity and chirality of ipsdienol, normalised by ln(Y+1) and $\arcsin\sqrt{Y}$, respectively (Schlyter and Birgersson 1989). Chirality data, transformed by arcsin(Y), for populations from Kimberley BC and Hat Creek CA were analysed by t test using the Minitab statistical package ver. 1.1 (Minitab Inc., State College PA). The coefficients of variation for the populations from Kimberley BC and Hat Creek CA were compared by t test performed by hand (Sokal and Braumann 1980).

3.1.1.3 Results

The mean quantities of ipsdienol per male varied between populations of *I. pini* (Table 10). Most of the variation was probably attributable to environmental factors and

Table 10. Quantities and chiralities of ipsdienol produced by individual male *I. pini* collected from eight localities in North America from 1984 to 1987.

	Quantity of ipsdienol (ng)			Chirality of ipsdienol [%(+)]			Correlation [a]		
Locality	N	Mean ± SE [b]	CV [c]	N	Mean ± SE	CV [d]	N	r	P
Pemberton BC	68	47 ± 15a	167.3%	14	65 ± 4.9	30.4%	14	-0.01	0.965
Williams Lake BC	75	158 ± 28b	93.7	38	71 ± 2.0	22.7	38	-0.02	0.896
Newcomb NY	110	165 ± 32b	78.8	55	57 ± 1.3	13.6	55	+0.49	<0.001
Osprey Lake BC	62	168 ± 34b	74.8	35	63 ± 2.7	25.8	35	-0.05	0.769
Radium BC	139	197 ± 21bc	66.0	87	52 ± 2.5	32.7	87	+0.18	0.105
Hat Creek CA	73	203 ± 33c	36.0	62	9 ± 2.1	19.0	62	-0.34	0.006
Princeton BC	457	315 ± 16cd	39.6	344	66 ± 1.0	33.1	344	-0.02	0.690
Kimberley BC	158	524 ± 41d	26.4	173	11 ± 1.4	19.8	142	-0.42	<0.001

[a] Pearson correlation coefficient between quantity and chirality of ipsdienol.

[b] Means followed by the same letter are not significantly different at P=0.05 [Scheffe's Multiple Comparison test on data transformed by ln(Y+1)].

[c] Data transformed by ln(Y+1).

[d] Data transformed by arcsin $\sqrt{Y}$.

differences in vigor rather than differential competitive pressures. Males from Kimberley BC were quick to bore into logs and produced copious amounts of frass while beetles from Pemberton BC and Newcomb NY were the least vigorous of all populations with respect to rates of boring and feeding. Geographic variation in brood host could have affected the ability of adult males to produce ipsdienol in lodgepole pine. New York beetles were bred in red pine, in contrast to beetles from the other seven localities which were bred in lodgepole pine. Lodgepole pine used as a brood host could also vary significantly between localities. Beetles from Kimberley and Radium BC were reared from logs of lodgepole pine with thicker phloem than that used by beetles from the rest of British Columbia.

Intrapopulation variation in the quantity of ipsdienol produced by individual males was found in all eight populations (Figs. 32A-H), with coefficients of variation ranging from 26.4 to 167.3% (Table 10). The high coefficient of variation for the New York population may have been a consequence of the change from red pine, as a brood host, to lodgepole pine for pheromone production. In all populations, most males contained low quantities of pheromone and relatively few contained large amounts. The frequency distributions and coefficients of variation are similar to those for the production of *trans*-verbenol by female *Dendroctonus ponderosae* (Borden et al. 1986; Hunt et al. 1986) and *cis*-verbenol and 2-methyl-3-buten-2-ol by male *Ips typographus* (Birgersson et al. 1984; Schlyter et al. 1987a; Birgersson et al. 1988; Schlyter and Birgersson 1989).

Interpopulation variation in the chirality of ipsdienol was evident. Males from Hat Creek CA and Kimberley BC produced primarily (-)-ipsdienol (Figs. 33A,B) with mean proportions of the (-) enantiomer of 9 and 11% of total ipsdienol, respectively (Table 10). The difference in mean chiralities in these two populations was only weakly significant (t test, df=104, P=0.096). As expected, males from Newcomb NY produced a little more (+)- than (-)-ipsdienol (Fig. 33C) with a mean proportion of 57% of the

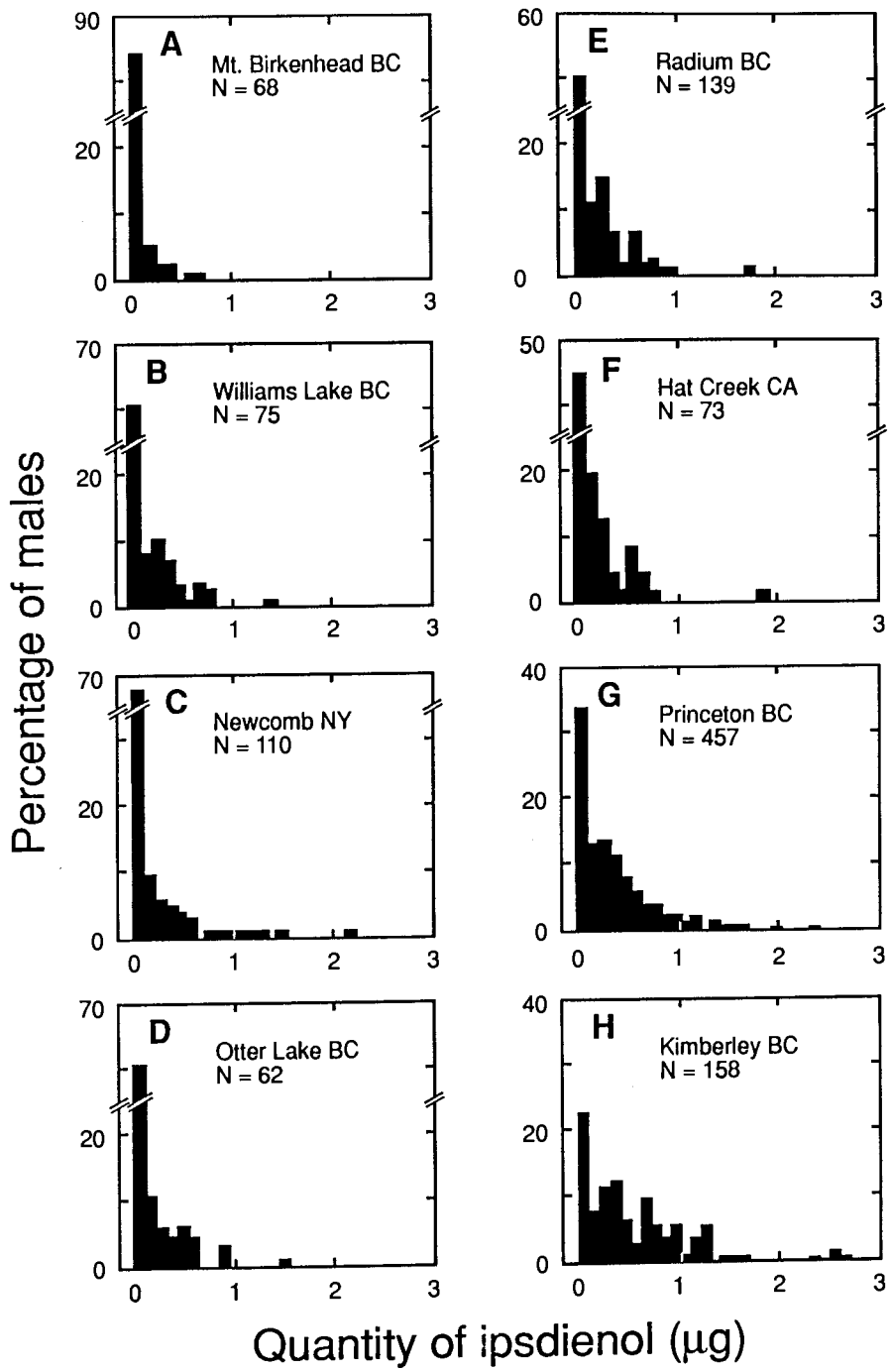


Figure 32. Frequency distributions of the quantities of ipsdienol produced by individual male *I. pini*.

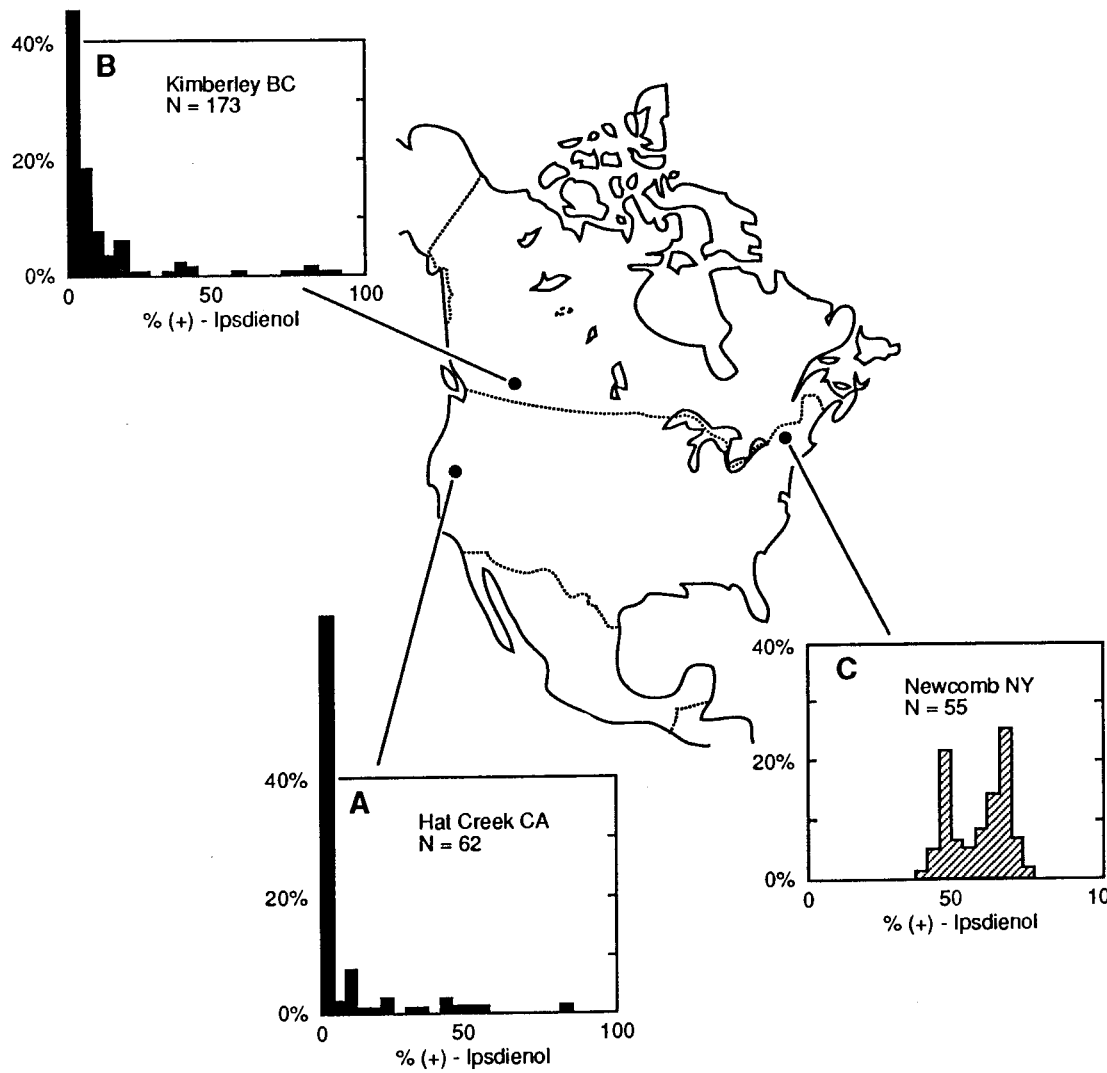


Figure 33. Frequency distributions of the chirality of ipsdienol produced by individual male *I. pini* from three populations in western (A and B) and eastern North America (C). Histograms shaded with different patterns are significantly different (see text for statistics).

(+)-enantiomer. The mean chiralities for all three of the above populations were reasonably close to the previously published determinations (Plummer et al. 1976; Birch et al. 1980a; Lanier et al. 1980).

However there was obvious interpopulation variation in the production of chiral ipsdienol by males from other populations in British Columbia (Figs. 34A-E). Males from the four most-western populations in British Columbia (Princeton, Pemberton, Williams Lake and Osprey Lake) produced more (+)- than (-)-ipsdienol (Figs. 34A-D) with mean proportions of the (+) enantiomer ranging from 63 to 71% of total ipsdienol (Table 10). There were no significant differences in the distributions of chiral ipsdienol production among these four populations (Chi^2 test, df=27, P=0.194). These populations were significantly different from the population from Kimberley BC (Chi^2 test, all df=9, all P<0.001). Males in the population from Radium BC produced almost equal amounts of both enantiomers; the mean proportion of (+) enantiomer was 52% of total ipsdienol (Table 10). The distribution of the ipsdienol chiralities in males from Radium BC was significantly different from that of the Kimberley population (Chi^2 test, df=9, P<0.001) and the remaining four populations from BC (Chi^2 test, all df=9, all P<0.001). These populations negate previous generalisations that western populations of *I. pini* are homogeneous with respect to the chirality of their aggregation pheromone.

Intrapopulation variation in the chirality of ipsdienol was found in all eight populations (Figs. 33A-C;34A-F) with coefficients of variation ranging from 13.6 to 33.1% (Table 10). The coefficients were comparable to that of the proportion of *cis*-verbenol in the total verbenol produced by male *I. typographus* (Birgerrson et al. 1988). There was no significant difference between the coefficients of variation for the populations from Kimberley BC and Hat Creek CA (t test, df=233, P>0.5). Variation in the modalities of the eight distributions of ipsdienol chiralities was also apparent. The distributions for the populations from Hat Creek CA and Kimberley BC had long tails but were strongly centered around a chiral ratio between 5:95 and 15:85 (+):(-), with

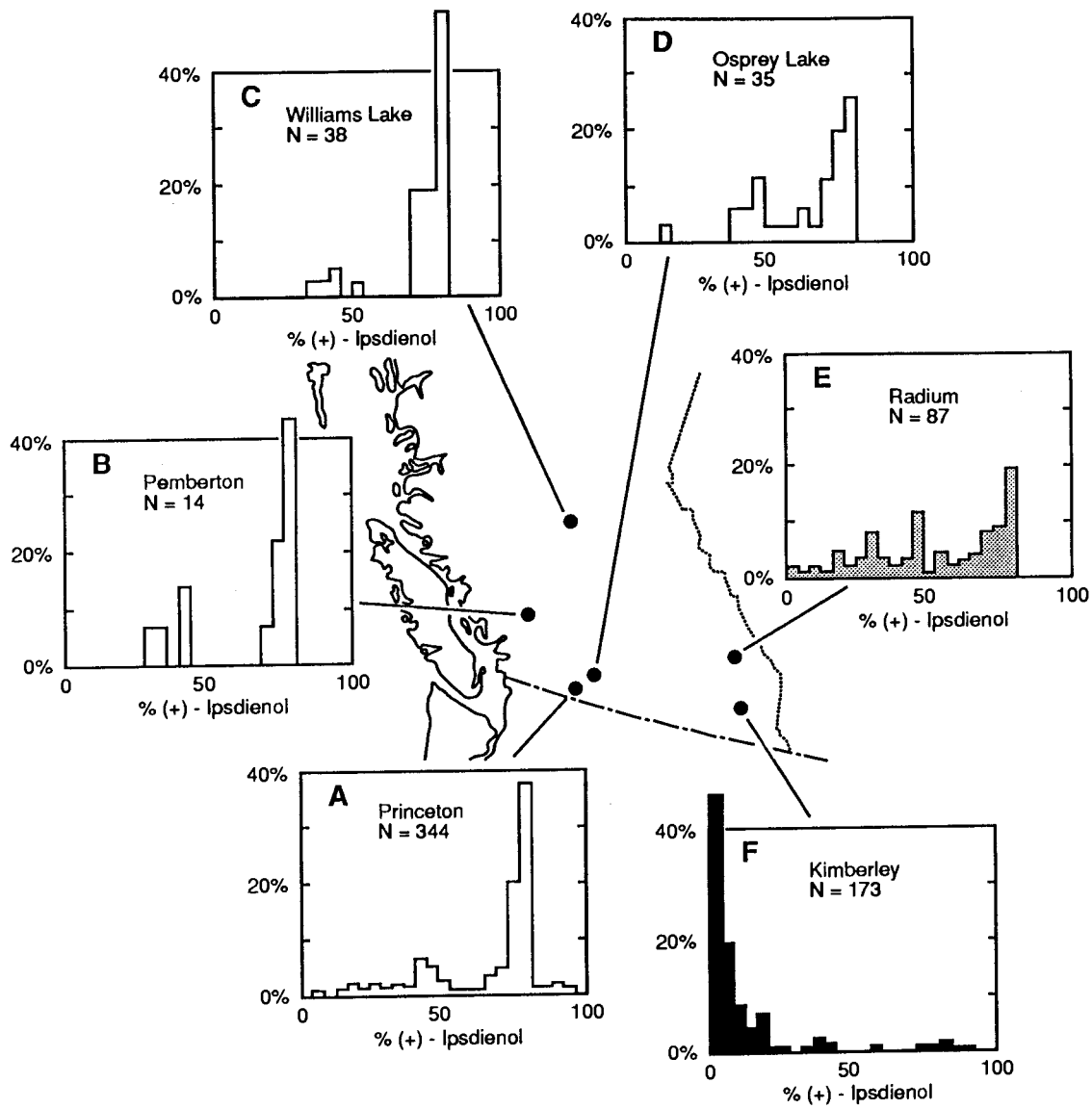


Figure 34. Frequency distributions of the chirality of ipsdienol produced by individual male *I. pini* from six populations in British Columbia. Histograms with different shading patterns are significantly different (see text for statistics).

only one mode in each. Bimodality was evident in the chiral distribution of the population from Newcomb NY, with both modes almost equal in size. In the populations from central- and south-western BC, the modes were distinctly different in size with a major mode between the ratios of 75:25 and 80:20 (+):(-). The chiral distributions from these populations were similar to that of the population from Newcomb NY in that all five have modes between 40:60 and 45:55 (+):(-). The earlier estimates from pooled samples (Stewart 1975; Plummer et al. 1976; Birch et al. 1980a; Lanier et al. 1980) failed to disclose these differences between individuals in the same population as well as the bimodality in some populations.

3.1.1.4 Discussion

For bark beetles, a pheromone message should contain reliable information regarding the qualities of either the host or the sender. Such a message should not be subject to chance variation or noise. In *I. pini*, I found that there is substantial variation in both quantity and chirality of ipsdienol. Most of the variation in quantity can probably be attributed to variation in vigor and environmental factors such as brood host and levels of precursors in the host tissue. Production of the pheromone, *cis*-verbenol, by male *I. typographus* increased directly with the concentration of vapors of the precursor, (-)-α-pinene (Byers 1981). In *I. typographus*, over 80% of the variation in quantities of *cis*-verbenol, *trans*-verbenol and myrtenol were explained by the variation in the amounts of α-pinene in the host (Birgersson et al. 1984). In addition, the quantity of pheromone in the hindgut may vary over time in the same individual. The rates of ingestion and defecation may not necessarily be constant.

The major factors responsible for the variation in chirality of ipsdienol were not the same as those responsible for the variation in quantity. No more than 25% of the variation in chirality of ipsdienol in any population was explained by the variation in the

quantity of ipsdienol (all $r^2<0.25$). In most populations in British Columbia less than 5% of the variation in the chirality of ipsdienol was explained by the variation in quantity of ipsdienol (all $r^2<0.05$). Since both enantiomers are produced from the same achiral precursor, myrcene (Hughes 1974; Byers et al. 1979; Renwick and Dickens 1979; Hendry et al. 1980; Byers 1981; Fish et al. 1984; Hunt et al. 1986), it seems unlikely that environmental factors should significantly affect the chirality of ipsdienol, certainly not to the extent seen in the quantity of ipsdienol. Variation in enzymatic composition due to genetic variation is the most probable source of the variation in chirality. It is quite likely that the production of ipsdienol of a specific chirality by an individual male *I. pini* is a quantitative genetic trait.

A major requirement for microevolution to occur, with respect to a quantitative trait, is the existence of significant levels of heritable variation. To date, heritability of pheromone quality has been clearly estimated only for the pink bollworm, *Pectinophora gossypiella*. Collins and Cardé (1985) found that the heritability of the sex pheromone blend of the (*Z,E*) and (*Z,Z*) isomers of 7,11-hexadecadienyl acetate, produced by female *P. gossypiella* from a laboratory strain, was 0.34. The variation of the chirality of ipsdienol in *I. pini* (CV range=13.6-33.1%) (Table 10) is more than that of the *E:Z* ratio of the sex pheromone in *P. gossypiella* (CV=5.3%). If there is a large heritable component to the variation in the chirality of ipsdienol produced by male *I. pini*, and sufficient selection pressure within a population, then such high levels of variation (Table 10) should facilitate microevolutionary changes in relatively-short periods of time, possibly giving rise to further geographic variation. Selection for female redbanded leafrollers, *Argyrotaenia velutinana*, producing high and low ratios of *(E)*- and *(Z)*-tetradecenyl acetate, resulted in indications of directional selection after only one generation of selection (Roelofs et al. 1986). The realised heritability was approximately 0.41 while the coefficient of variation was only 16.3%.

Geographic variation in the production of chiral ipsdienol by *I. pini* is consistent with the hypothesis that selection pressures involved in the maintenance of reproductive and ecological isolation shape the patterns of pheromone use in communities. *Ips pini* and *I. paraconfusus* are broadly sympatric in California (Bright and Stark 1973). Both breed in fallen ponderosa pines in spring and early summer (Birch 1978). However, they rarely breed in the same material at the same time. There is some evidence that interspecific competition among brood of these two species is greater than intraspecific competition (Light et al. 1983). The pheromone for *I. pini* in this region is (-)-ipsdienol (Stewart 1975; Birch et al. 1980a; Fig. 33A) while male *I. paraconfusus* produce primarily (+)-ipsdienol, in conjunction with ipsenol and *cis*-verbenol (Silverstein et al. 1966; Wood et al. 1968). The pheromones of these two species are mutually inhibitory (Birch and Wood 1975; Birch and Light 1977; Birch et al. 1977; Birch 1978; Light and Birch 1979; Birch et al. 1980a; Byers 1989). In other areas of the western United States, where *I. paraconfusus* is absent, *I. pini* is broadly sympatric with *I. hoppingi* and *I. confusus*, two sibling species of *I. paraconfusus* (S.L. Wood 1982). The pheromones of these three sibling species are probably quite similar since all three are cross-attracted to each others' pheromones (Lanier and Wood 1975; Cane et al. 1989,1990).

The population in Kimberley BC is associated with a forest type that includes a significant proportion of ponderosa pine along with lodgepole pine, similar to that in areas in northern California. The similarity in production profiles of chiral ipsdienol between populations from Kimberley BC and Hat Creek CA (Figs.33A and B) suggest that the Kimberley population is probably derived from populations similar to that of Hat Creek. Competitors similar to *I. paraconfusus*, such as *I. hoppingi* and *I. confusus*, are not present in Kimberley BC (Bright 1976). In the absence of any other selection pressure, stabilising selection should exert its influence, resulting in a normalised distribution around the same mean. However, there is no significant difference in coefficients of variation between the population from Kimberley and that from Hat Creek

(t test, df=233, P>0.5) and the difference in mean chiralities is only weakly significant (t test, df=104, P=0.096). Both populations are skewed (g_1 = -2.819 and -2.504, respectively) and leptokurtic (g_2 = 7.764 and 6.476, respectively). The predicted effect of stabilising selection may have been countered by genetic influx from adjacent, southern populations were competitors do exert pressure.

Post-glaciation colonisation and random drift are probably responsible for the patterns found in New York and most of British Columbia. I suggest that the eastern populations of *I. pini* originated from a southeastern refugium in the Appalachians. In the west, populations with production profiles much like those from Princeton and Williams Lake, invaded western North America from a southwestern refugium. It is unlikely that populations found in areas, such as Princeton and Williams Lake, originated from Beringia. After the Cordilleran ice sheet retreated, colonisers from a southwestern refugium would have had to halt their advance at the southernmost edge of the Cordilleran (the edge as found during glaciation) to allow *I. pini* originating from Beringia to colonise. Moreover, there is no evidence that *I. pini* was in refuge in Beringia (S.L. Wood 1982). *Ips pini* has been collected from only two localities in Alaska, both at the southern tip of the state (S.L. Wood 1982).

It is possible that the differences between production profiles for populations from areas such as Princeton and Williams Lake and areas such as New York could have arisen as a consequence of random drift while the populations were separated either during or after Wisconsin glaciation. Alternatively, differences could have arisen after colonisation as a consequence of competition pressures. However, there is no evidence that competitors similar to *I. paraconfusus* are present in either British Columbia or eastern North America. Nor is there any information regarding the production profile for the ancestral population of *I. pini*. If character displacement in the chirality of ipsdienol produced by male *I. pini* occurred, due to either reproductive or ecological isolation, then whence was it displaced?

The population from Radium BC may be an intergrade between the two principle western population types. The lack of a major peak near 0% on the (+)-ipsdienol scale of the profile is further evidence of a quantitative trait. If the trait was governed by a simple Mendelian system then we would expect a proportion of both major production types in the population following equilibrium.

Heritable variation in quantitative traits is probably maintained by a balance between mutation pressure and stabilising selection (Slatkin and Kirkpatrick 1986). Unless there are major genes involved in the trait (Roelofs et al. 1986), it is unlikely that mutation could introduce bimodality into a population. Bimodality in the populations from Newcomb NY and most of British Columbia suggests that other selection pressures are currently exerting their influences in these areas. These pressures may not be stable over microevolutionary time such as the rotation period of host trees. The modes may separate further and possibly result in behavioral isolation and subsequent speciation, or converge and lead to greater homogeneity. Alternatively, bimodality may be stable in these populations (Mather 1955), representing mixed evolutionary stable strategies (Smith 1982) in which individuals from both modes have equal fitness, on average. The causative agents are simply not known, but may involve sexual selection, reproductive isolation and interspecific competition (Baker and Cardé 1979; Roelofs 1980; Cardé and Baker 1984; West-Eberhard 1984; Cardé 1986). A full understanding of the patterns of geographic variation in production should be facilitated with information on the geographic variation in behavioral responses to chiral ipsdienol, as well as information on the association between production and response traits.

3.1.2 Inter- and intrapopulation variation in the response of *Ips pini* to chiral ipsdienol

3.1.2.1 Objective and Hypotheses

In California, where male *I. pini* produce primarily (-)-ipsdienol, beetles are attracted by (-)-ipsdienol but repelled by ipsdienol with as little as 5% (+) enantiomer (Stewart 1975; Birch et al. 1980a). In New York, where males produce a 65:35 mixture of (+)- and (-)-ipsdienol, beetles respond best to a racemic mixture (Lanier et al. 1980). Geographic variation in the production of chiral ipsdienol is as divergent among populations in British Columbia as between populations from California and New York (Chap. 3.1.1).

My objectives were twofold. Firstly, I planned to describe the inter- and intrapopulation variation in the response of *I. pini* to chiral ipsdienol. Secondly, I planned to determine if there was an obvious association between production and response traits. I tested the four following hypotheses: 1) the proportions of a population of *I. pini* responding to ipsdienol of different chiralities (response profile) would not be homogeneous in any population; 2) the response profiles for the populations from Williams Lake and Princeton would not differ; 3) the response profile for the population from Kimberley would differ from those of Williams Lake and Princeton; and 4) the response profile for the population from Radium would be intermediate between the profile for the population from Kimberley and those for the populations from Williams Lake and Princeton.

3.1.2.2 Materials and Methods

For Experiment 1, (±)-ipsdienol was obtained from Borregaard, A.S., Sarpsborg, Norway and had a chemical purity >95%. Chiral ipsdienols [98% (+) and 98% (-)]

were obtained from E.K. Czyzewska (Department of Chemistry, Simon Fraser University, Burnaby BC) and each had chemical purities > 98%. For Experiments 2-4, (±)-ipsdienol and chiral ipsdienols [98% (+) and 98% (-)] (chemical purities, 98, 93 and 83%, respectively) were obtained from PheroTech Inc. (Delta BC).

In Experiment 1, each lure consisted of ten Microcap® disposable pipettes (2 µL) (Drummond Scientific Co., Broomall PA), each sealed at one end and filled with ipsdienol, and placed in a polyethylene, micro-centrifuge tube (1.8 mL) (Evergreen Scientific, Los Angeles CA). The release of ipsdienol with different chiral ratios was achieved by adjusting the relative proportion of tubes filled with either (±)-ipsdienol or one of the enantiomers (98% chiral purity). The release rate of ipsdienol from each lure was approximately 100 µg/day at 24 °C (determined by weight loss).

In Experiments 2-4, each lure consisted of a 10-cm-length of C-flex® tubing (ID=1.6 mm; OD=2.4 mm) (Concept Inc., Clearwater FL), filled with an ethanol solution of ipsdienol (80 mg/mL). Different solutions were prepared for each of 11 different chiral ratios of ipsdienol. The release rate of ipsdienol from each lure was approximately 0.6 mg/day at 24 °C (determined by collection of volatiles on Porapak-Q).

In all experiments, replicates of twelve 8-unit, multiple-funnel traps (Lindgren 1983) (PheroTech Inc., Delta BC) were set in grids of 3 x 4 in stands of lodgepole pine. Replicate grids were placed at least 100 m apart, and traps were spaced 10-15 m apart within each replicate. Each trap was baited, and suspended such that the top of each trap was approximately 1.3-1.5 m above ground level. No trap was within 2 m of any tree.

Experiments 1-4 determined the effect of chiral ipsdienol on the attraction of *I. pini* near Princeton, Williams Lake, Radium and Kimberley BC, respectively. Ten replicates were set for Experiments 1-3 during the periods of 22 May to 9 June, 1986; 5 July to 6 Sept, 1987; and 7 July to 9 Sept, 1987, respectively. Eight replicates were set

for Experiment 4 between 13 July and 6 Sept, 1989. Eleven different chiral ratios of ipsdienol and a control were the 12 treatments, randomly assigned within each replicate. In Experiment 1, the control did not have any lure while in Experiments 2-4, the control contained C-flex® ethanol lures. All lures were replaced, and treatments re-randomised within each replicate, at intervals of 3-4 weeks.

Trap catch data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). Data, transformed by ln(Y+1) to remove heteroscedasticity, were analysed by three-way analysis of variance (ANOVA) using site, treatment, replicate nested within site, and the interaction between site and treatment as model factors. For each site, data were analysed by two-way ANOVA using replicate and treatment as model factors, first with all treatments and secondly with only the ipsdienol treatments. Eleven and ten orthogonal contrasts were conducted for the first and second ANOVA's for each site, respectively, comparing the control against each ipsdienol treatment separately in the first case and comparing the response to (±)-ipsdienol against each of the remaining ipsdienol treatments separately in the second. Sex-ratio data [transformed by arcsin(Y)], for catches equal to or greater than ten, were subjected to full-factorial two-way ANOVA. For each site, untransformed sex-ratio data (not means), for catches equal to or greater than ten, were regressed against the chirality of ipsdienol, using treatment as the only factor in a general linear model. Tests of homogeneity of slopes were performed by analysis of covariance (ANCOVA) using site and the interaction between site and the covariate, chirality of ipsdienol, as model factors. One contrast was performed, comparing the slope for the population in Kimberley against the slope for the remaining populations. For each site, I determined the Pearson correlation coefficient between mean proportional responses to each of eleven chiral ratios and the associated mean proportion of males in populations producing the same chiral ratio (±5 %) as determined in Chap. 3.1.1.

3.1.2.3 Results

Ips pini showed considerable variation in response to chiral ipsdienol (Figs. 35A-D). Catches of *I. pini* to ipsdienol-baited traps were not homogeneous with respect to chirality (Table 11). In all populations, significantly-fewer beetles were caught in traps baited with 98% (+)-ipsdienol than in traps baited with (±)-ipsdienol (Figs. 35A-D). Significantly-fewer beetles were caught in traps baited with 2% (+)-ipsdienol than in traps baited with (±)-ipsdienol in Williams Lake, Radium and Princeton BC (Figs. 35A-C). Except for one contrast, significantly-more beetles were caught in all traps at all locations baited with ipsdienol than in control traps (orthogonal contrasts, ANOVA, all $P<0.029$). The exception was in Kimberley where the increase in catches to traps baited with 98% (+)-ipsdienol, relative to controls, was only weakly significant (orthogonal contrast, ANOVA, $P=0.079$).

The relative, chiral-specific responses of *I. pini* within a population (response profile) to ipsdienol varied between locations (Table 11). The effect of the interaction between location and treatment on trap catches was still significant even when the Kimberley population was removed from the analysis [ANOVA, $F(30,319)$, $P<0.001$].

The sex ratio of captured *I. pini* varied among chirality treatments (Table 11). In all populations, the proportion of males caught in ipsdienol-baited traps increased as the proportion of (+)-ipsdienol increased (Figs. 36A-D). The pattern of variation in sex ratio varied between locations (Table 11). However, the interaction between location and treatment did not have a signif ant effect on sex ratio when the Kimberley population was omitted from the analysis [ANOVA, $F(18,200)$, $P=0.646$]. The slopes of the regression lines for these three populations were significantly different from that of the Kimberley population [orthogonal contrast, ANCOVA, $P=0.003$].

The association between the mean chiral-specific responses of *I. pini* to ipsdienol, relative to the mean total response of *I. pini* in a population, and the relative frequency

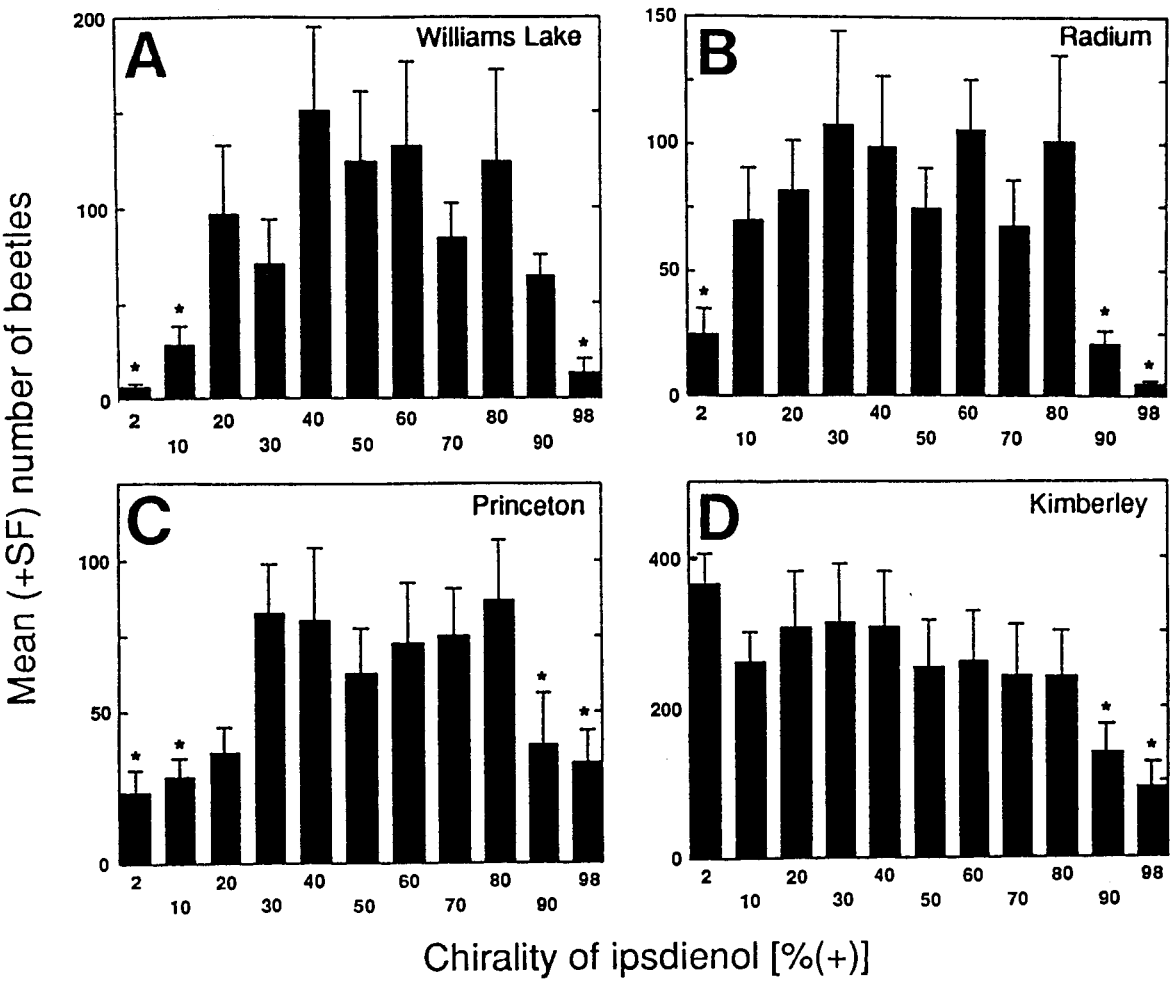


Figure 35. The effect of chiral ipsdienol on the capture of *I. pini* in multiple-funnel traps near Williams Lake (A), Radium (B), Princeton (C) and Kimberley BC (D) (n=10, 10, 10 and 8, respectively). Some treatments (*) were significantly different from the 50% (+) treatment (orthogonal contrasts, ANOVA, all $P<0.08$).

Table 11. Analysis of variance (ANOVA) on the effects of location (Williams Lake, Radium, Princeton and Kimberley BC), chirality of ipsdienol [2-98% (+)] and replicate (n=8 or 10) nested within location, on the number and sex ratio of *I. pini* captured in ipsdienol-baited multiple-funnel traps.

	Trap catch [a]			Proportion of males [b]		
Source	df	F	P	df	F	P
Location (A)	3	86.95	<0.001	3	309.81	<0.001
Chirality (B)	10	33.98	<0.001	10	11.67	<0.001
Replicate nested within site (C)	34	15.81	<0.001	34	4.54	<0.001
A * B	30	5.77	<0.001	28	1.68	0.021
Error	319			260		

[a] Data transformed by ln(Y+1).

[b] Data transformed by arcsin(Y).

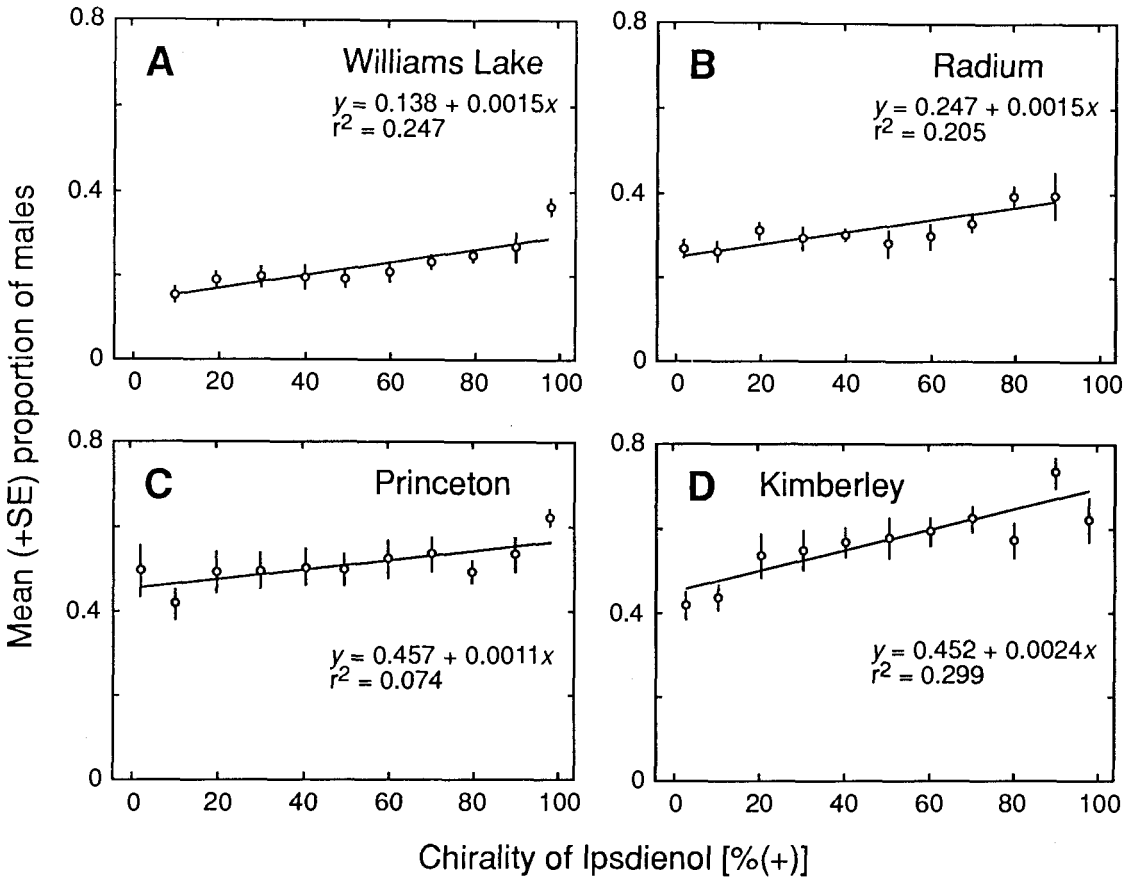


Figure 36. The effect of chiral ipsdienol on the sex ratio of *I. pini* captured in multiple-funnel traps near Williams Lake (A), Radium (B), Princeton (C) and Kimberley BC (D) (N=82, 79, 78 and 97, respectively). The slopes of the regression lines are significantly different from zero (t tests, all $P<0.01$).

of production of ipsdienol of the associated chirality (± 5%) by males for populations was variable (Figs. 37A-D). In Radium and Princeton the correlations were significantly different from zero (Table 12). The correlation was only weakly significant for the population from Kimberley and not significant for the population from Williams Lake. In no case did the variation in one variable explain more than 50% of the variation in the other variable (all $r^2<0.475$).

3.1.2.4 Discussion

I found significant heterogeneity in the responses of *I. pini* to chiral ipsdienol in all four populations in British Columbia (Figs. 35A-D). In no case was there evidence of enantio-specific responses to ipsdienol as shown by *I. pini* in California (Birch et al. 1980a). The relative responses of *I. pini* to chiral ipsdienol should reflect relative benefits to individuals. The broad range of equal response levels in most populations should, therefore, reflect equal fitnesses for responding individuals. However, fitness estimations for specific tactics should consider that these response profiles may mask several different behavioral traits. Responses of an individual may vary in accordance with a set probability function. Cardé et al. (1976) determined the intrapopulation variation in the response of male oriental fruit moths, *Grapholita molesta*, to synthetic pheromone blends. Individual male moths were marked with fluorescent powders and recaptured using the same pheromone blends. Initial preferences shown by individuals were not associated with their subsequent preferences. Yet the response profiles for the population were the same on both occasions. Such probability functions may be determined, in part, by state parameters as age and vigor, as well as by genetics. Secondly, the shape of these probability functions may vary between individuals due to differences in variance or types of curves. Male *G. molesta* and male pink bollworm moths, *Pectinophora gossypiella*, exhibit a high degree of pheromone-blend specificity at an ambient

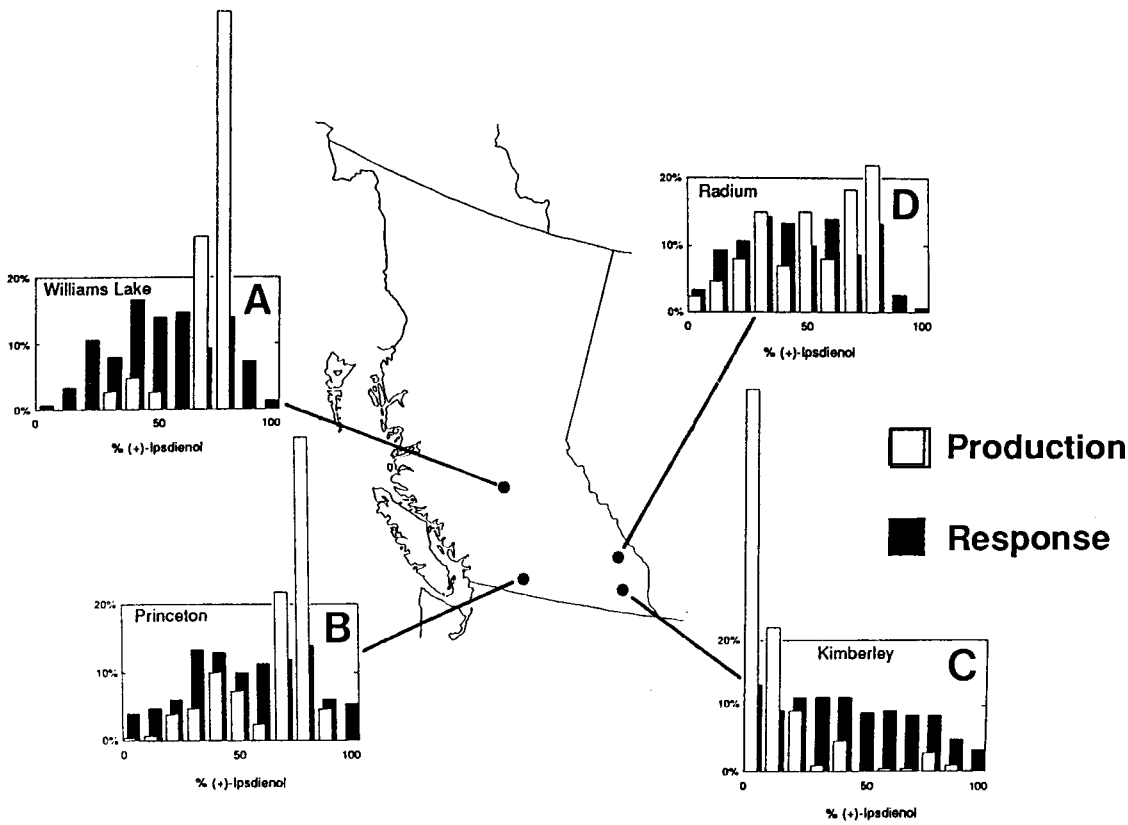


Figure 37. The associations between the production of chiral ipsdienol by male *I. pini* and the response of male and female *I. pini* to chiral ipsdienol, near Williams Lake (A), Princeton (B), Kimberley (C) and Radium BC (D).

Table 12. Correlations between the production of chiral ipsdienol by male *I. pini* and the response of male and female *I. pini* to chiral ipsdienol in four localities in British Columbia.

Locality	Pearson correlation coefficient (r)	P
Williams Lake	0.356	0.283
Kimberley	0.538	0.088
Princeton	0.624	0.040
Radium	0.689	0.019

temperature of 20 °C (Linn et al. 1988). When tested at 26 °C, however, males of both species exhibited a significantly-lower degree of specificity. And lastly, the preferred chiral ratio used to set the probability function may vary between individuals. All three traits may have some genetic components.

The evidence of dose-dependent responses with respect to sex ratio should reflect differential benefits to males and females. In Kimberley, females show a stronger preference for ipsdienol with low proportions of the (+) enantiomer. This trend is consistent with the profile of total response in the population and production of chiral ipsdienol by males. However it is not clear why this trend, *albeit* at a lower intensity, should be evident in the other three populations. It is possible that it is a sex-linked trait which is residual from other selection pressures, or possibly a trait that cannot vary in a non-linear fashion due to genetic constraints.

Beetles in the Kimberley population differ from the remaining three populations, with respect to their behavioral responses to chiral ipsdienol. The response of *I. pini* to ipsdienol with chiralities ranging from 2% to 20% (+), relative to the response to 50% (+), decreased in the populations from Williams Lake, Princeton and Radium but not in the population from Kimberley (Figs. 35A-D). Secondly, the sex-specific responses of *I. pini* were more intense in the Kimberley population relative to the other three populations (Figs. 36A-D).

These similarities support my hypothesis that populations from Williams Lake, Princeton and Radium are more closely related to each other than to the population from Kimberley BC. The strong response to 2-20% (+)-ipsdienol by *I. pini* in Kimberley, and the similarity in production profiles (Figs. 33A,B), are evidence of relatedness between populations from southeastern British Columbia and California. The lack of specificity in the responses by *I. pini* in the Kimberley population is consistent with the removal of selection pressures from a population where competition has influenced the use of semiochemicals. *Ips paraconfusus* is not present in Kimberley

BC. The population response profile has broadened, relative to that of populations from California, to include most of the possible chiralities of ipsdienol produced by male *I. pini*, so much so that the production and response traits are only weakly correlated (Table 12).

Three conclusions arise from my data. Firstly, the population profile for the response of individual bark beetles to a signal may not necessarily be predicted by the population profile for the transmission of a signal. The response profiles for beetles in Kimberley and Williams Lake BC were broad but differed from the production profiles (Figs. 37B,D). The correlations between production and response were not strongly significant in either locations (Table 12).

Secondly, pheromone-mediated behavioral separation may not necessarily be a premating isolation mechanism leading to speciation in bark beetles. In Kimberley BC, there was a lack of enantio-specificity in the response of *I. pini* to ipsdienol (Fig. 35D), even though selection for enantio-specificity in the use of ipsdienol as a communication channel was evidenced by the strong enantio-specificity in the production of ipsdienol (Fig. 33B). It is probable that if two strains of *I. pini*, differing only in enantio-specificity in the use of ipsdienol, were to evolve in allopatry, then hybridisation would occur in sympatry. Two distinct strains (so-called *E* and *Z* strains) of the European corn borer, *Ostrinia nubilalis*, occur in Europe and North America, differing in the relative use of *(E)*- and *(Z)*-11-tetradecenyl acetates as pheromones (Kochansky et al. 1975; Klun and Cooperators 1975; Anglade et al. 1984; Barbattini et al. 1984; Peña et al. 1988; Löfstedt 1990). Hybridisation of the two strains occurs in areas where they are sympatric (Roelofs et al. 1985; Cardé 1986; Klun and Huettel 1988).

Thirdly, there is considerable intrapopulation variation in the response of *I. pini* to chiral ipsdienol. Individuals in a population should not be expected to all do the same thing. Bark beetles are major economic pests of forestry (Furniss and Carolin 1980) and pheromones are gaining acceptance as pest management tools. If pheromones

are used to mass trap *I. pini*, would 'resistance' to pheromone baits occur? Lanier et al. (1972) suggested that some populations of *I. pini* are already 'resistant' to a single pheromone blend. Since the levels of variation of ipsdienol chirality were high in all four populations of *I. pini* (Table 10), I hypothesise that 'resistance' to a pheromone blend could develop within a population as well. The development of 'resistance' has been shown in laboratory colonies of the khapra beetle, *Trogoderma granarium*. After 18 generations of selection for non-response by males, there was a 74% reduction in mean response by males to the natural pheromone produced by female beetles (Rahalkar et al. 1985). The possibility exists that populations of bark beetles, such as *I. pini*, subjected to repeated use of pheromone-based trapping programs using a fixed pheromone blend, could develop 'resistance' by shifting to another blend (Lanier et al. 1972; Lanier and Burkholder 1974). Knowledge of the variation and heritability in the response to pheromones should help to predict the consequences of artificial selection pressures such as mass trapping.

4.0 SUMMARY AND CONCLUSIONS

Species-specificity and geographic variation are two community-based patterns often associated with reproductive and ecological isolation. My data provide evidence that both patterns occur with respect to the use of semiochemicals by bark beetles. I hypothesise that interspecific reproductive interference, interspecific competition, random drift and post-glaciation colonisation are the major factors involved in the evolution of these patterns.

Species-specificity in the use of semiochemicals occurs among three sympatric species of bark beetles, *Ips latidens*, *I. pini* and *Dendroctonus ponderosae*, in stands of lodgepole pine in British Columbia. The concept of species-specificity infers that random events are not responsible for the pattern, thereby suggesting directed selection in the evolution of the traits. Temporally-separated kairomone-, pheromone- and synomone-based behaviors have the potential to contribute to reproductive and ecological isolation among these three species. However, some of these patterns may have been incidental, a consequence of random events or not valid for rejection of the hypothesis. Firstly, the three species exhibit some temporal separation in their semiochemical-mediated flight periods. Flight periods are probably a consequence of developmental periods, which in turn are probably influenced by resource quality. It is unlikely that interspecific pressures influenced flight periods directly, although these pressures could have some indirect effect. It is probably coincidental, therefore, that temporal separation aids in reproductive and ecological isolation. Secondly, species-specificity in the use of pheromones was exhibited by the three species. *Ips latidens* and *I. pini* used ipsenol and ipsdienol, respectively, as pheromones while *D. ponderosae* used *exo*-brevicomin with *cis*- and *trans*-verbenol. My data do not refute the hypothesis that species-specificity is a general occurrence in a community of bark beetles. However, they do not support the hypothesis either. The choice of species was not random, having been determined with *a*

priori knowledge that different pheromones were used by the three species. An appropriate test of the hypothesis would have required a random selection of three species.

On the other hand, the species-specific patterns of the use of kairomones and synomones by these three species cannot reasonably be ascribed to random chance. The probability that seven common monoterpenes found in lodgepole pine (selected without *a priori* knowledge) should affect the behavior of three species is low (P=0.010). The probability that the four pheromones (ipsenol, ipsdienol, *exo*-brevicomin and verbenol mixture) used by the three species should have activity in six of the seven possible interactions is also quite low (P=0.055). Furthermore, the probability that all six of these actions would also be inhibitory is even lower (P<0.001). And finally, dose-dependent inhibition or attraction to kairomones, pheromones or synomones occurred in 22 of 29 test cases. This trend is further evidence that non-random events are at work since it is probable that the hardware (receptor cells) and software required (central neural system) for such responses would be quite considerable.

If the pattern of species-specificity in the use of semiochemicals is not random, then which factors are the most probable causes of the pattern? None of my data was designed to test specific hypotheses. However circumstantial evidence suggests that these patterns are a consequence of individuals of all three species minimising interspecific reproductive interference and interspecific competition. Species-specificity in the response of individuals to pheromone producers, due to the effect of mutual inhibition caused by these same semiochemicals as synomones, and in their response to monoterpenes as kairomones, can ensure that conspecifics aggregate on the same patch. The phenomenon of mass aggregation is most probably a consequence of interspecific competition. Monoterpene levels are indicative of patch quality, measured in terms of nutritional quality, moisture conditions and host defense mechanisms. Reproductive isolation is also assured by these same mechanisms since mass aggregations usually

consist only of conspecifics. The relative contributions of interspecific reproductive interference and interspecific competition to species-specificity are difficult to isolate and require carefully-designed experiments.

If selection pressures from interspecific interactions are responsible for the community structure in pheromone use, then the use of a pheromone by one species should vary as a consequence of variation in levels of interspecific interactions. In most of the western United States, *I. pini* is sympatric with competitors, such as *I. paraconfusus*, and exhibits ecological and reproductive isolation from them. The pheromone for *I. pini* in this region is (-)-ipsdienol while male *I. paraconfusus* produce primarily (+)-ipsdienol, in conjunction with ipsenol and *cis*-verbenol. The pheromones of these two species act as synomones, and are mutually inhibitory.

Additional factors may be involved in determining the remaining geographic variation for *I. pini*. Post-glaciation colonisation and random drift are probably responsible for the patterns found in New York and most of British Columbia. I hypothesise that the eastern populations of *I. pini* originated from a southeastern refugium while western populations originated a southwestern refugium. Differences between populations such as Princeton BC and Newcomb NY probably arose as a consequence of random drift while the populations were separated either during or after Wisconsin glaciation. Patterns of character displacement in populations such as that from Hat Creek CA probably arose more recently. There is no evidence of competitors in the other regions.

Details on proximate causes and effects provide advantages in understanding the evolutionary genetics of invertebrate behavior (Dethier 1986) and should lead to the formation of biologically tenable and testable models of evolution (Bush 1986). My thesis may provide a framework for further studies on the evolution of the use of semiochemicals with particular reference to interspecific competition and speciation.

5.0 APPENDIX

Table 13. List of pine species (Coniferales: Pinaceae) cited in text.

Common Name	Species
Loblolly pine	*Pinus taeda* Linnaeus
Lodgepole pine	*Pinus contorta* var. *latifolia* Engelmann
Ponderosa pine	*Pinus ponderosa* Douglas ex Lawson and Lawson
Red pine	*Pinus resinosa* Aiton
Western white pine	*Pinus monticola* Douglas

Table 14. List of insect species cited in text.

Species	Order[a]: Family	Common Name
Amorbia cuneana (Walsingham)	Lep.: Tortricidae	western avocado leafroller
Argyrotaenia velutinana (Walker)	Lep.: Tortricidae	redbanded leafroller
Ctenopseustis obliquana (Walker)	Lep.: Tortricidae	brownheaded leafroller
Dendroctonus frontalis Zimmermann	Col.: Scolytidae	southern pine beetle
Dendroctonus ponderosae Hopkins	Col.: Scolytidae	mountain pine beetle
Dendroctonus pseudotsugae Hopkins	Col.: Scolytidae	Douglas-fir beetle
Dendroctonus terebrans (Olivier)	Col.: Scolytidae	black turpentine beetle
Gnathotrichus retusus (LeConte)	Col.: Scolytidae	
Gnathotrichus sulcatus (LeConte)	Col.: Scolytidae	
Grapholita molesta (Busck)	Lep.: Tortricidae	oriental fruit moth
Ips avulsus (Eichhoff)	Col.: Scolytidae	small southern pine engraver
Ips calligraphus (Germar)	Col.: Scolytidae	
Ips confusus (LeConte)	Col.: Scolytidae	
Ips emarginatus (LeConte)	Col.: Scolytidae	
Ips grandicollis (Eichhoff)	Col.: Scolytidae	
Ips hoppingi Lanier	Col.: Scolytidae	
Ips latidens LeConte	Col.: Scolytidae	
Ips mexicanus (Hopkins)	Col.: Scolytidae	
Ips paraconfusus Lanier	Col.: Scolytidae	California fivespined ips
Ips pini (Say)	Col.: Scolytidae	pine engraver
Ips typographus Linnaeus	Col.: Scolytidae	
Ostrinia nubilalis (Hubner)	Lep.: Pyralidae	European corn borer
Pectinophora gossypiella (Saunders)	Lep.: Gelechiidae	pink bollworm
Pityogenes chalcographus (Linnaeus)	Col.: Scolytidae	six-spined spruce bark beetle
Pityogenes plagiatus knechteli Swaine	Col.: Scolytidae	
Platyptila carduidactyla (Riley)	Lep.: Pterophoridae	artichoke plume moth
Platyptila williamsii Grinnell	Lep.: Pterophoridae	
Tomicus piniperda Linnaeus	Col.: Scolytidae	
Trogoderma granarium Everts	Col.: Dermestidae	khapra beetle
Trypodendron lineatum (Olivier)	Col.: Scolytidae	striped ambrosia beetle

[a] Lepidoptera (Lep.) or Coleoptera (Col.).

Table 15. List of scolytid pheromones and synomones cited in text.

Common Name	IUPAC Name
exo-Brevicomin	(+)/(-)-*exo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane
endo-Brevicomin	(+)/(-)-*endo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane
Ipsdienol	*R*-(-)/*S*-(+)-2-methyl-6-methylene-2,7-octadien-4-ol
Ipsenol	*R*-(+)/*S*-(-)-2-methyl-6-methylene-7-octen-4-ol
Lineatin	3,3,7-trimethyl-2,9-dioxatricyclo[3.3.1.$0^{4,7}$]nonane
Myrtenol	4,6,6-trimethylbicyclo[3.1.1]hept-3-en-10-ol
Sulcatol	6-methylhept-5-en-2-ol
cis-Verbenol	*R*/*S*-(+)/(-)-*cis*-4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol
trans-Verbenol	*R*/*S*-(+)/(-)-*trans*-4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol
Verbenone	4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one

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