IPSENOL: AN AGGREGATION PHEROMONE FOR Ips latidens (LECONTE) (COLEOPTERA: SCOLYTIDAE)

DANIEL R. MILLER,1,*, JOHN H. BORDEN,1 G.G.S. KING,2 and KEITH N. SLESSOR2

1Centre for Pest Management
Department of Biological Sciences
Canada V5A 1S6
2Department of Chemistry
Simon Fraser University
Burnaby, British Columbia, Canada V5A 1S6

(Received January 9, 1991; accepted March 25, 1991)

Abstract—Ipsenol was identified from the frass of male, but not female, Ips latidens from British Columbia, feeding in phloem tissue of lodgepole pine, Pinus contorta var. latifolia. The responses of I. latidens to sources of ipsenol and cis-verbenol were determined with multiple-funnel traps in stands of lodgepole pine in British Columbia. Ipsenol attracted both male and female I. latidens, verifying that it is a pheromone for this species. Male I. latidens showed a slight preference for (S)-(-)-ipsenol. cis-Verbenol was not produced by beetles of either sex and, in contrast to an earlier report, both enantiomers inhibited attraction to ipsenol-baited traps. The predators, Enoclerus sphenaxis and Thanaisinus undatus (Cleridae), were attracted to traps baited with cis-verbenol and ipsenol.

Key Words—Pheromone, ipsenol, cis-verbenol, chirality, Ips latidens, Coleoptera, Scolytidae, predator, kairomone, Enoclerus sphenaxis, Thanaisinus undatus, Cleridae.

INTRODUCTION

In British Columbia, the bark beetle, Ips latidens (LeConte) (Coleoptera: Scolytidae), feeds and breeds in the phloem tissue of lodgepole and ponderosa pines, Pinus contorta var. latifolia Engelmann and P. ponderosa Douglas ex Lawson

*To whom correspondence should be addressed at: Phero Tech Inc., 7572 Progress Way, RR #5, Delta, British Columbia, Canada V4G 1E9.

1517

0003-4843/91/0100-1517$03.00 © 1991 Picture Publishing Corporation
and Lawson, respectively (Bright, 1976; Furniss and Carolin, 1980; Wood, 1982). Like many other bark beetles, *I. latidens* has the potential to be a significant pest in stands of lodgepole pine, particularly in association with the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Scolytidae), or during periods of chronic drought (Furniss and Carolin, 1980; Miller and Borden, 1985).

Ipsenol (2-methyl-6-methylene-7-octen-4-ol) has been implicated as a pheromone for *I. latidens* in California. *Ips latidens* were caught, albeit in low numbers, on traps baited with either ipsenol or a mixture of ipsenol and *cis*-verbenol (*cis*-4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol) (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, the question of whether ipsenol is a pheromone for *I. latidens* is still unresolved since the production of ipsenol by *I. latidens* has not yet been determined.

Our objective was to determine the identity of pheromone(s) used by *I. latidens* in stands of lodgepole pine in British Columbia. Various scolytid species show behavioral responses to different enantiomers of ipsenol and the related chiral alcohol, ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) (Borden, 1982). Therefore, we 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. Concurrent research by Seybold et al. (1991) tested similar hypotheses for *I. latidens* in California, breeding in ponderosa pine.

**METHODS AND MATERIALS**

In 1984, adult *I. latidens* were obtained from a 2-year-old colony that originated near the east gate of Manning Park, British Columbia. Using the gelatin-pill-capsule technique (Borden, 1967), 16 adult males and five adult females were restrained, individually, on noninfested bolts of lodgepole pine, collected near Princeton, British Columbia. Beetles were allowed to bore into the bark and feed for 24 hr. The frass of each individual was crushed in 150 μl of pentane. These extracts were analyzed by splitless capillary gas chromatography (Hewlett Packard HP 5890 using a 30-m × 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).

Racemic ipsenol (chemical purity, 98%) was obtained from Bedoukian
Research Inc. (Danbury, Connecticut). B.J. Johnston (Department of Chemistry, Simon Fraser University, Burnaby, British Columbia) supplied chiral ipsenols (optical purities, 96% (S)-(−) and 94% (R)-(+) respectively; chemical purities, 98%). (−)-β-Phellandrene was obtained from the SCM Corporation (Jacksonville, Florida).

Phero Tech Inc. (Vancouver, British Columbia) supplied polyethylene, bubble-cap devices containing the following chemicals: (1) racemic ipsenol (chemical purity, 98%) in solution with 1,3-butadiene; (2) 1,3-butadiene (chemical purity, >98%); (3) ethanol (chemical purity, 99%); and (4) chiral cis-verbenols (optical purities, 84% (S)-(−) and 94% (R)-(+) respectively; chemical purities, 98%).

β-Phellandrene was released from closed, polyethylene, microcentrifuge tubes (1.8 ml) (Evergreen Scientific, Los Angeles, California). The release rate was approximately 8 mg/day at 27–30°C (determined by weight loss). Ipsenol release devices consisted of either 10-cm lengths of C-flex tubing (OD = 1.6 mm; ID = 2.4 mm) (Concept Inc., Clearwater, Florida) filled with a solution of ipsenol in ethanol, or polyethylene, bubble-cap devices filled with a solution of ipsenol in 1,3-butadiene, 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). 1,3-Butadiene was not released from either C-flex or bubble-cap lures. Ethanol release devices consisted of either 10-cm lengths of C-flex tubing or polyethylene bubble-caps, each filled with ethanol and heat-pressure sealed. The release rates of ethanol from these devices were approximately 10 and 6 mg/day, respectively, at 24°C (determined by weight loss). cis-Verbenol was released from polyethylene, bubble-cap devices at a rate of 3–6 mg/day at 27–30°C (determined by weight loss).

In all experiments, eight-unit, multiple-funnel traps (Lindgren, 1983) (Phero Tech) were set in mature stands of lodgepole pine near Princeton, British Columbia. 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 assigned randomly within replicates. Sexes of *I. latidens* captured in experiment 1 were determined by dissection and examination of genitalia. Sexes of *I. lati-
dens* captured in other experiments were not determined due to insufficient numbers for most of the treatments. Sexes of other beetles caught in traps were not determined.

In experiments 1–3, 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 × 3, from May 23 to July 2, 1987. The treatments, using C-flex devices, were as follows: (1) blank control; (2) ethanol control; (3) racemic ipsenol (0.6 mg/day); (4) racemic ipsenol (1.2 mg/day); (5) (S)-(−)-
ipsenol (0.6 mg/day); and (6) (R)-(+)-ipsenol (0.6 mg/day). All ipsenol devices contained ethanol.

Experiment 2 tested the effects of ethanol and the interaction between ipsenol and ethanol. Seven replicates of four traps per replicate were set in grids of 2 x 2, from August 6 to 31, 1989. The treatments, using bubble-cap devices, were as follows: (1) 1,3-butanediol; (2) ethanol and 1,3-butanediol (as two separate devices); (3) racemic ipsenol; and (4) racemic ipsenol and ethanol (as two separate devices). All ipsenol devices contained 1,3-butanediol.

Experiment 3 tested for interaction between (S)-(−)-cis-verbenol and the combination of ipsenol and β-phellandrene. β-Phellandrene is used as a kairomone by L. latidens (Miller and Borden, 1990). Nine replicates of four traps per replicate were set in grids of 2 x 2, from May 21 to June 23, 1988. The treatments, using C-flex devices, were as follows: (1) ethanol control; (2) racemic ipsenol and β-phellandrene (as two separate devices); (3) (S)-(−)-cis-verbenol; and (4) the combination of racemic ipsenol, β-phellandrene, and (S)-(−)-cis-verbenol (as three separate devices). All ipsenol devices contained ethanol.

Experiment 4 tested for interaction between (R)-(−)-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 along parallel trap lines, spaced 50 m apart, from June 21 to July 10, 1989. The treatments, using bubble-cap lures, were as follows: (1) 1,3-butanediol; (2) racemic ipsenol; (3) (R)-(−)-cis-verbenol; and (4) racemic ipsenol and (R)-(−)-cis-verbenol (as two separate devices). All ipsenol devices contained 1,3-butanediol.

The data were analyzed using the SAS statistical package version 5.0 (SAS Institute Inc., Cary, North Carolina). Trap catches of all species were transformed by ln(Y + 1) to remove heteroscedasticity. Sex ratio data for L. latidens were normalized by an arcsin transformation. Homoscedastic data were subjected to either one-, two-, or three-way analysis of variance (ANOVA). Evidence of synergy in the attraction of beetles, due to the interaction of multiple components, was determined by the interaction term in ANOVA. Two multiple contrasts were performed in experiment 1. Ryan-Einot-Gabriel-Welsch (REGW) multiple-range tests were used in experiments 2–4 when P < 0.05.

RESULTS AND DISCUSSION

Ipsenol is a pheromone for L. latidens. It was found in the frass of 10 of 16 male L. latidens (estimated range, 10 ng to 1 mg), 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
racemic isopenol by gas chromatography. *cis*-Verbénol was not found in any samples. Similar results were found by Seybold et al. (1991) for Californian *I. latidens*. The major monoterpene in the frass was β-phellandrene. β-Phellandrene is the major monoterpeno in the phloem tissue of lodgepole pine (Mirov, 1961; Shrimpton, 1972, 1973) and is a kairomone for *I. latidens* (Miller and Borden, 1990).

In experiments 1 and 2, *I. latidens* were significantly attracted to isopenol, with a slight preference for (S)-(-)-isopenol (Figures 1A and 2). The results in

Fig. 1. The effect of chiral isopenol on the number (A) and sex ratio (B) of *Ips latidens* responding to multiple-funnel traps near Princeton, British Columbia, in experiment 1 from May 23 to July 2, 1987 (N = 11). Mean numbers grouped by a line are significantly different from the blank and ethanol controls as well as (S)-(-)-isopenol [multiple contrasts, F(1,49), P < 0.001 and P = 0.025, respectively, on data transformed by ln(Y + 1)]. Mean proportions of males grouped by a line are significantly different from (S)-(-)-isopenol [multiple contrast, F(1,30), P = 0.008, on data transformed by arcsin(Y)]. Some treatments (*) had insufficient numbers for determinations of sex ratios.

Fig. 2. The effect of ethanol and isopenol on the attraction of *Ips latidens* to multiple-funnel traps near Princeton, British Columbia, in experiment 2 from August 6 to September 2, 1989 (N = 7). Means followed by the same letter are not significantly different at P = 0.05 [REGW multiple range test on data transformed by ln(Y + 1)].
experiment 1 can be attributed solely to ipsenol, since ethanol alone was not attractive and there was no significant interaction between ethanol and ipsenol in experiment 2 [ANOVA, F(1.24), P = 0.441 and P = 0.989, respectively]. The sex ratios of *I. latidens* captured in experiment 1 were affected by chirality [ANOVA, F(3,30), P = 0.048]. Proportionally more males responded to (5)-(-)-ipsenol than to either racemic or (R)-(+) -ipsenol (Figure 1B). Our results agree with the field data of Wood et al. (1967) and Furniss and Livingston (1979) and recent laboratory data of Seybold et al. (1991).

In contrast to results from California (Wood et al., 1967), both enantiomers of cis-verbenol inhibited the response of *I. latidens* to sources of ipsenol (Figures 3 and 4). cis-Verbenol was not produced by male *I. latidens*. cis-Verbenol is produced by sympatric species of bark beetles such as *D. ponderosa* (Pierce et al., 1987; Libbey et al., 1985) and may act as a synomone

**Fig. 3.** The effect of (5)(−)-cis-verbenol and the combination of ipsenol and β-phellandrene on the attraction of *Ips latidens* to multiple-funnel traps near Princeton, British Columbia, in experiment 3 from June 8 to 23, 1988 (N = 9). All treatments contained ethanol. Means followed by the same letter are not significantly different at P = 0.05 [REGW multiple range test on data transformed by ln(Y + 1)].

**Fig. 4.** The effect of (R)-(−)-cis-verbenol and ipsenol on the attraction of *Ips latidens* to multiple-funnel traps near Princeton, British Columbia, in experiment 4 from June 21 to July 10, 1989 (N = 10). Means followed by the same letter are not significantly different at P = 0.05 [REGW multiple range test on data transformed by ln(Y + 1)].
(Nordlund and Lewis, 1976), facilitating resource partitioning and minimizing interspecific competition for phloem tissue. The role of synomones in cross-attraction and host partitioning has been demonstrated in bark beetle communities such as the loblolly pine, *P. taeda* L., community of southern pine beetles in the southern United States (Hedden et al., 1976; Vité et al., 1978; Dixon and Payne, 1979; Birch et al., 1980; Sviha et al., 1980; Paine et al., 1981; Watterson et al., 1982).

Sources of ipsenol and/or cis-verbolenol and/or ethanol also were attractive to other species of bark beetles. In experiment 1, *Ips* *mexicanus* (Hopkins) preferred traps baited with ipsenol, regardless of chirality or release rate (Table 1). The treatment with the highest release rates of ipsenol and ethanol were preferred by *Hylurgops porosus* (LeConte) and a *Pityophthorus* Eichhoff species. Unlike the *Pityophthorus* species, *H. porosus* was attracted to ipsenol alone. *Hylastes longicollis* Swaine did not exhibit any preferences in experiment 1. However, in experiment 3, ipsenol with β-phellandrene was the preferred treatment for *H. longicollis* as well as for *I. mexicanus* and a *Pityophthorus* species (Table 2). *Hylurgops porosus* showed equal preference for the combinations of ipsenol and β-phellandrene, and ipsenol with β-phellandrene and (S)-(−)-cis-verbolenol.

**Table 1. Effects of Chiral Ipenol on Attraction of Ips mexicanus, Hylastes longicollis, Hylurgops porosus, and a Pityophthorus sp. (Scolytidae) to Multiple-Funnel Traps near Princeton, British Columbia, in Experiment 1, May 23 to July 2, 1987 (N = 11)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Ips mexicanus</em></th>
<th><em>Hylastes longicollis</em></th>
<th><em>Hylurgops porosus</em></th>
<th><em>Pityophthorus</em> species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control</td>
<td>0.1 ± 0.1 a</td>
<td>6.3 ± 1.4</td>
<td>12.9 ± 4.2 a</td>
<td>5.7 ± 3.1 a</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.1 ± 0.1 a</td>
<td>8.7 ± 1.8</td>
<td>23.0 ± 5.3 bc</td>
<td>4.0 ± 1.9 a</td>
</tr>
<tr>
<td>Ethanol + (R)/(+) 1 ipsenol (0.6 mg/day)</td>
<td>2.8 ± 1.1 b</td>
<td>7.9 ± 1.2</td>
<td>25.3 ± 4.6 bc</td>
<td>7.4 ± 2.1 ab</td>
</tr>
<tr>
<td>Ethanol + racemic 1 ipsenol (0.6 mg/day)</td>
<td>2.5 ± 0.7 b</td>
<td>6.3 ± 1.3</td>
<td>22.8 ± 5.1 bc</td>
<td>7.8 ± 1.8 ab</td>
</tr>
<tr>
<td>Ethanol + racemic 1 (1.2 mg/day)</td>
<td>2.4 ± 0.9 b</td>
<td>9.0 ± 1.7</td>
<td>31.7 ± 4.9 c</td>
<td>13.3 ± 3.7 b</td>
</tr>
<tr>
<td>Ethanol + (S) (−) 1 ipsenol (0.6 mg/day)</td>
<td>2.5 ± 0.9 b</td>
<td>5.8 ± 1.4</td>
<td>19.2 ± 4.0 b</td>
<td>3.3 ± 0.8 a</td>
</tr>
</tbody>
</table>

*Means within a column followed by different letters are significantly different at P = 0.05 [REGW multiple range test on data transformed by In(Y + 1)].

*No significant differences among means [ANOVA, F(5, 49), P = 0.232].
Table 2. Effects of (S)-(−)-cis-Verbenol and Combination of Racemic Ispenol and β-Phellandrene on Attraction of *Ips mexicanus, I. perturbatus, Hylastes longicollis, Hylurgops porosus*, a *Pityphorus Species* (Scolytidae), and *Enoclerus sphageus* (Cleridae), to Multiple-Funnel Traps near Princeton, British Columbia, in Experiment 3, June 8–23, 1988 (N = 7)

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Ips mexicanus</em></th>
<th><em>Ips perturbatus</em></th>
<th><em>Hylastes longicollis</em></th>
<th><em>Hylurgops porosus</em></th>
<th><em>Pityphorus species</em></th>
<th><em>Enoclerus sphageus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>0.2 ± 0.2 a</td>
<td>0.8 ± 0.5 a</td>
<td>3.8 ± 0.6 a</td>
<td>9.0 ± 3.5 a</td>
<td>0.8 ± 0.3 a</td>
<td>0.7 ± 0.7 a</td>
</tr>
<tr>
<td>Ethanol + (S)-(−)-cis-verbenol</td>
<td>2.0 ± 0.6 b</td>
<td>1.4 ± 0.9 a</td>
<td>5.1 ± 1.8 a</td>
<td>8.6 ± 1.7 a</td>
<td>1.3 ± 0.4 a</td>
<td>5.3 ± 1.6 bc</td>
</tr>
<tr>
<td>Ethanol + Ispenol + β-Phellandrene</td>
<td>18.9 ± 3.5 c</td>
<td>1.4 ± 0.8 a</td>
<td>11.4 ± 2.5 b</td>
<td>21.3 ± 3.0 b</td>
<td>11.1 ± 3.0 b</td>
<td>1.8 ± 0.7 ab</td>
</tr>
<tr>
<td>Ethanol + Ispenol + β-Phellandrene + (S)-(−)-cis-verbenol</td>
<td>3.3 ± 0.5 b</td>
<td>1.9 ± 3.7 b</td>
<td>3.9 ± 1.2 a</td>
<td>14.6 ± 3.9 ab</td>
<td>2.1 ± 1.2 a</td>
<td>15.0 ± 5.1 c</td>
</tr>
</tbody>
</table>

*Means within a column followed by different letter are significantly different at P = 0.05 [REGW multiple-range tests on data transformed by ln(Y + 1)].
Explanations for significant treatment differences in these species must be viewed as speculative. Responses by bark beetles suggest that ipsenol and/or cis-verbolen are used as either pheromones or synomones (Tables 1 and 2). However, there are no data on the phenology of pheromone production by any of these species. The low numbers caught in traps, relative to *L. latidens*, may be a consequence of four factors: (1) low population numbers; (2) missing pheromones; (3) other semiochemical functions; or (4) random chance.

The experimental areas were selected for abundance of *L. latidens*. We have no data on the abundance of the other species. However, *I. pini* (Say) and *Pityogenes knechti* Swaine were very abundant in both years but neither was trapped. Ispenol inhibits the response of *I. pini* to suitable hosts and to its own pheromone, ipsdienol (Birch and Light, 1977; Birch et al., 1977; Furniss and Livingston, 1979). It seems reasonable to hypothesize that ipsenol and/or cis-verbolen are also inhibitory to *P. knechti*, or have no effect.

The preferred treatment for the clerid (Coleoptera), *Enoclerus sphaegeus* F., in experiment 3 was the combination of ipsenol with ethanol, β-phellandrene and (5S)(−)-cis-verbolen (Table 2). The interaction between treatments had an additive, not synergistic, effect on the attraction of *E. sphaegeus* [ANOVA, *F*(1,24), *P* = 0.822]. In experiment 4, both *E. sphaegeus* and *Thanasimus undatus* Say (Cleridae) preferred the combination of ipsenol and (R)-(−)-cis-verbolen over all other treatments (Table 3). The interaction between ipsenol and (R)-(−)-cis-verbolen had a synergistic effect on the attraction of *E. sphaegeus* [ANOVA, *F*(1,36), *P* = 0.001] but an additive effect on the attraction of *T. undatus* [ANOVA, *F*(1,36), *P* = 0.560].

**Table 3. Effects of (R)-(−)-cis-Verbolen and Racemic Ispenol on Attraction of *Enoclerus sphaegeus* and *Thanasimus undatus* (Cleridae) to Multiple-Funnel Traps near Princeton, British Columbia, in Experiment 4, June 21 to July 10, 1989 (N = 10)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Enoclerus sphaegeus</em></th>
<th><em>Thanasimus undatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control</td>
<td>0.1 ± 0.1 a</td>
<td>0.1 ± 0.1 a</td>
</tr>
<tr>
<td>(R)-(−)-cis-Verbolen</td>
<td>0.2 ± 0.1 a</td>
<td>2.3 ± 1.0 b</td>
</tr>
<tr>
<td>Ispenol</td>
<td>1.2 ± 0.3 b</td>
<td>2.3 ± 0.5 b</td>
</tr>
<tr>
<td>Ispenol + (R)-(−)-cis-verbolen</td>
<td>5.9 ± 0.9 c</td>
<td>6.0 ± 1.1 c</td>
</tr>
</tbody>
</table>

*Means within a column followed by different letters are significantly different at *P* = 0.05 [REGW multiple range tests on data transformed by In(Y + 1)].
Responses by the clerids, *E. sphageus* and *T. undatulus*, to sources of cis-verbenol and the combination of isopentol and cis-verbenol (Tables 2 and 3) are consistent with other studies demonstrating the use of bark beetle pheromones as kairomones by predators (Borden, 1982; Dahlsten, 1982). The lack of specificity in prey of *E. sphageus* is exemplified by its capacity to respond to pheromones of other species such as *exo-brevicomin* produced by *Dendroctonus* and *Dryocoeters* species (Borden et al., 1987).

Acknowledgments—We thank D.L. Wood and S.J. Scyboid for their encouragement, collaboration and reviews of the manuscript. Additional comments were received from two anonymous reviewers. D.E. Bright and E. Rickey kindly verified the identifications of *Scolytidae* and *Ceridae*, respectively. Voucher specimens have been deposited with the Entomology Museum at Simon Fraser University. G. Owen provided assistance with gas chromatography and spectral analyses, and B.J. Johnston resolved both enantiomers of isopentol. L. Wheeler, T. Richerson, and C. Matteau assisted in processing captured beetles. Assistance in the manufacture of isopentol release devices was provided by Pherotech Inc. This research was supported in part by an H.R. MacMillan Family Fund Fellowship and a Simon Fraser University Graduate Research Fellowship to D.R.M., the Natural Sciences and Engineering Research Council of Canada (Operating Grant A3881 and Strategic Grant G1611), and the Science Council of British Columbia [Grant 1 (RC 14-16)].

REFERENCES


