THE USE OF MONOTERPENES AS KAIROMONES BY IPS LATIDENS (LECONTE) (COLEOPTERA: SCOLYTIDAE)

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Abstract

The responses of Ips latidens (LeConte) to multiple-funnel traps baited with various monoterpenes were determined in stands of lodgepole pine in British Columbia. β-Phellandrene was attractive to I. latidens in the absence of the pheromone ipsenol. β-Phellandrene increased the attraction of I. latidens to ipsenol-baited traps while α-pinene, 3-carene, terpinolene, and myrcene reduced trap catches. Differential responses by males and females to sources of ipsenol with or without β-phaellandrene suggest that the information conveyed to males differs from that conveyed to females. Species-specific responses to monoterpenes by I. latidens and three sympatric species of scolytids suggest that unique combinations of kairomones could be important in facilitating matchings with conspecifics. Pheromones need not be the sole constituent of species-specific chemical messages.

Résumé

La réponse d’Ips latidens (LeConte) à divers monoterpenes placés dans des pièges à entonnoirs multiples a été déterminée dans des peuplements de pin lodgepole en Colombie britannique. β-Phellandréine a attiré I. latidens en absence de la phéromone ipsénohol. β-Phellandréine a accru l’attraction de I. latidens vers des pièges contenant ipsénohol alors que α-pinèine, 3-careène, terpinolène et myrcène ont réduit les captures. Des niveaux de réponse différents pour mâles et femelles à des sources d’ipsénohol, avec ou sans β-phellandréine, suggèrent que l’information transmise aux mâles diffère de celle transmise aux femelles. L’existence de réponses aux monoterpenes spécifiques à l’espèce, pour I. latidens et trois espèces sympatriques de scolytides, suggèrent que des combinaisons uniques de kairomones pourraient être importantes dans la facilitation de l’accouplement avec des individus co-spicifiques. Les phéromones ne sont pas nécessairement les seules composantes des messages chimiques spécifiques à l’espèce.

Introduction

In British Columbia, the bark beetle, Ips latidens (LeConte), feeds and breeds in the phloem tissue of lodgepole and ponderosa pines, Pinus contorta var. latifolia Engelmann and P. ponderosa Douglas ex Lawson, respectively (Bright 1976; Furniss and Carolin 1980; Wood 1982). Ips latidens aggregates on suitable hosts in response to the male-produced pheromone ipsenol (2-methyl-6-methylene-7-octen-4-0) (Wood et al. 1967; Furniss and Livingston 1979; S.L. Seybold personal communication; unpublished data). As well, primary attraction of I. latidens to lodgepole pine has been demonstrated in British Columbia, although the kairomones were not identified (Miller et al. 1986). Beetles preferentially landed on trees stressed by girdling of the phloem tissue at a height of 3.5–4.0 m above ground. Monoterpenes are abundant in phloem tissue of lodgepole pine (Shrimpton 1972, 1973) and are used as kairomones by scolytids in genera other than Ips (Borden 1982; Byers et al. 1985; Byers et al. 1988; Chénier and Philogene 1989; Dickens et al. 1984; Schroeder 1988; Schroeder and Eidmann 1987; Schroeder and Lindelow 1989; Volz 1988). It seems probable, therefore, that monoterpenes may also play a role as kairomones for I. latidens. Because β-phellandrene is the most abundant monoterpen in phloem of lodgepole pine, it should be the most likely candidate.

Our objective was to test the hypothesis that in stands of lodgepole pine in British Columbia, monoterpenes found in the phloem tissue of lodgepole pines, particularly β-phellandrene, are used as kairomones by I. latidens.

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Materials and Methods

Chemicals. (+)-3-Carene, (−)-β-phellandrene, racemic α-pinene, (−)-β-pinene, and terpinolene (chemical purities, all >95%) were obtained from H.D. Pierce, Jr. (Department 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 Pherotech Inc. (Vancouver, B.C.). Racemic isopin (chemical purity, 98%) was obtained from Bedoukian Research Inc. (Danbury, CT).

Release Devices. Monoterpenes were released from closed, polyethylene micro-centrifuge tubes (400 μL) (Evergreen Scientific, Los Angeles, CA), each filled with a single monoterpane. 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 per day, respectively, at 27°C (determined by weight reduction). An isopin 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 isopin in ethanol, or plain ethanol (99%) for ethanol controls, and heat-pressure sealed at both ends. The release rate of isopin was approximately 0.6 mg per day at 24°C (determined by collection of volatiles on Porapak-Q).

Trapping Experiments. 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, B.C. 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.

The effects of various monoterpenes in combination with isopin were tested in Experiment 1. Ten replicates of nine traps per replicate were set in grids of 3 × 3, from 24 May to 2 July in 1987. The treatments were as follows: (1) blank control; (2) ethanol control; (3) racemic isopin; (4) isopin with 3-carene; (5) isopin with myrcene; (6) isopin with β-phellandrene; (7) isopin with α-pinene; (8) isopin with β-pinene; and (9) isopin with terpinolene.

Experiment 2 compared the effect of β-phellandrene with that of the combination of the other five monoterpenes, with and without isopin. Ten replicates of eight traps each were set in grids of 2 × 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) racemic isopin alone; (6) isopin with β-phellandrene; (7) isopin with 3-carene, myrcene, α-pinene, β-pinene, and terpinolene; and (8) isopin with all six monoterpenes.

Statistical Analyses. The data were analyzed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary, NC). When necessary, trap catch data were transformed to remove heteroscedasticity. In Experiment 1, catches of I. latidens, Hylastes longicollis Swaine, and I. mexicanus (Hopkins) were transformed by ln(Y + 1). In Experiment 2, catches of I. latidens were transformed by ln(Y) and those of I. mexicanus were transformed by ln(Y + 1). Sex ratio and all remaining catch data were not transformed. Homoscedastic data were subjected to one-way ANOVA and Duncan’s multiple range test when P < 0.05. In Experiment 1, a priori multiple contrasts were also performed on the trap catch data for I. latidens, I. mexicanus, H. longicollis, and Hylurgops porosus LeConte. In Experiment 2, three-way full-factorial ANOVA was performed to determine interaction effects.

Results

Experiment 1. Isopin and Monoterpenes. Monoterpenes had a significant effect on the attraction of I. latidens to sources of isopin (Fig. 1). The combination of β-phellandrene and isopin was the preferred treatment, increasing trap catches by >100% relative to isopin alone. Multiple contrasts discerned groupings in the data. The catches in traps baited with isopin and either 3-carene, myrcene, α-pinene, or terpinolene seemed to be
Fig. 1. The effects of various monoterprenes on the attraction of *Ips laitensis* to ipinol-baited multiple-funnel traps near Princeton, B.C., 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 log10).

Similar as a group, their trap catches were compared with that of traps baited with ipinol alone and the reduction was found to be weakly significant (multiple contrast ANOVA, *P*=0.074). There was no significant difference in the sex ratio of *I. laitensis* among the different treatments [ANOVA, *F*(7,49), *P*=0.922]. The mean (±SE) proportion of males in traps baited with ipinol was 0.16±0.010, significantly different from a 1:1 ratio (*t*-test, *P*<0.001, df=69).

*Hyalurgops porosus*, *H. longicollis*, and *I. mexicanus* showed significant differences due to treatments [ANOVA, *F*(8,80), *P*=0.020, *P*=0.001, and *P*<0.001, respectively]. *β*-Phellandrene and 3-carene increased catches of all three species (Table 1). *Hylastes longicollis* showed a strong preference for traps baited with 3-carene (Table 1). Terpinolene

### Table 1. The effect of monoterprenes on the attraction of *Ips mexicanus*, *Hyalurgops porosus*, and *Hylastes longicollis* to ipinol-baited multiple-funnel traps, near Princeton, B.C., 25 May to 2 July 1987 (N=10)

<table>
<thead>
<tr>
<th>Treatments</th>
<th><em>Ips mexicanus</em></th>
<th><em>Hyalurgops porosus</em></th>
<th><em>Hylastes longicollis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control</td>
<td>0±0.2</td>
<td>11±3.1</td>
<td>6±1.7</td>
</tr>
<tr>
<td>Ethanol control</td>
<td>0±0.2</td>
<td>17±4.5</td>
<td>6±0.9</td>
</tr>
<tr>
<td>Ipinol</td>
<td>3±1.1</td>
<td>16±4.6</td>
<td>4±0.9</td>
</tr>
<tr>
<td>Ipinol + <em>α</em>-pinene</td>
<td>5±1.6</td>
<td>15±3.8</td>
<td>3±0.8</td>
</tr>
<tr>
<td>Ipinol + myrcene</td>
<td>2.6±0.5</td>
<td>15±3.2</td>
<td>7±0.8</td>
</tr>
<tr>
<td>Ipinol + <em>β</em>-pinene</td>
<td>5±1.3</td>
<td>15±3.3</td>
<td>11±1.6</td>
</tr>
<tr>
<td>Ipinol + terpinolene</td>
<td>2±0.5</td>
<td>24±7.5</td>
<td>10±1.8</td>
</tr>
<tr>
<td>Ipinol + <em>β</em>-phellandrene</td>
<td>23±3.2</td>
<td>37±6.5</td>
<td>8±1.2</td>
</tr>
<tr>
<td>Ipinol + 3-carene</td>
<td>16±3.1</td>
<td>31±6.7</td>
<td>32±4.9†</td>
</tr>
</tbody>
</table>

*Means within a column grouped by a line are significantly different from the ipinol treatment at *P*<0.001 unless otherwise noted (multiple contrasts ANOVA, *F*(1,71) on data transformed by log10).

†Significantly different from the group of *Ips mexicanus*, *Ips mexicanus* + *β*-pinene, *Ips mexicanus* + terpinolene, and *Ips mexicanus* + *β*-phellandrene (*P*<0.001).
increased catches of *H. porosus* and *H. longicollis* relative to traps baited with ipsenol alone; the later species showed attraction to sources of myrcene and β-pinene as well. Only *I. mexicanus* showed any attraction to sources of ipsenol alone.

**Experiment 2. Ipsenol and β-Phellandrene.** *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. 2A). 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. Sources of the five-terpene mix of 3-carene, myrcene, α-pinene, β-pinene, and terpinolene were not attractive and inhibited responses of *I. latidens* to ipsenol, β-phellandrene, and the combination of ipsenol with β-phellandrene. Inhibition by the five-terpene mix is consistent with the weak inhibition demonstrated by four of the five monoterpenes in Experiment 1 (Fig. 1).

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 five-terpene mix than to β-phellandrene (Fig. 2B). 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 five-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.

*Ips mexicanus* was attracted, albeit in low numbers (all trap catches <10), to sources of β-phellandrene and ipsenol (ANOVA, *F*(1,71), *P* <0.001 and *P* <0.001, respectively); the preferred treatment was the combination of β-phellandrene and ipsenol (mean ± SE = 26 ± 13.0) (Duncan’s multiple range test, *P* <0.05). The interaction between ipsenol and β-phellandrene was additive, not synergistic (ANOVA, *F*(1,71), *P* = 0.196).
Discussion

Our results support the hypothesis that host kairomones are used by *I. latidens* (Fig. 1). β-Phellandrene was attractive alone and increased attraction to sources of ipsenol (Fig. 2A). In contrast, the four other monoterpene negated the effects of β-phellandrene (Fig. 2A). The combination of all six monoterpene with ipsenol significantly reduced trap catches relative to traps baited with ipsenol alone. These results are the clearest demonstration that monoterpene are used as kairomones by a species of *Ips* since monoterpene were implicated as attractants for *I. typographus* L. (Rudinsky et al. 1971a, 1971b; Tomescu et al. 1979) and *I. grandicollis* (Eichhoff) (Werner 1972a, 1972c). 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 phloem drier than that used by *I. pini* (Miller and Borden 1985). β-Phellandrene is the major monoterpene in phloem of lodgepole pine (Shirpinton 1972, 1973). As phloem dries, therefore, it would be the most likely to remain above the threshold level required for perception at some distance from a potential host. In fresh hosts, other monoterpene 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 monoterpene (Fig. 2B). 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* Hopkins (Furniss and Schmitz 1971) and *Tomiscus piniperda* (L.) (Byers et al. 1985) do not show sexual specificity in their responses to monoterpene. Kairomone probably 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 response to ipsenol-baited traps (Fig. 2B). This sex-specificity in responses to ipsenol does reflect differential benefits to females and males, as in the six-spined spruce bark beetle, *Pityogenes chalcographus* L., 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 monoterpene, 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 because 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* Lanier and *P. chalcographus* (Byers 1983; Byers et al. 1988). Schlyter et al. (1987) found that significantly more female than male *I. typographus* (L.) 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 pheromone probably signifies that some males are still looking for females. Pheromone production by males of three polygynous species, *I. paraconfusus*, *I. grandicollis* (Eichhoff), and *I. calligraphus* (Germar), seems to decrease as males acquire females, and apparently ceases as harem are filled (Borden 1967; Werner 1972b; Södra 1982).
Table 2. The relative effects of monoterpenes on the attraction of four sympatric species of Scolytidae in stands of lodgepole pine near Princeton, B.C. Repellency signified by −; no effect by 0; mild attraction by +; and strong attraction by ++

<table>
<thead>
<tr>
<th>Monoterpene</th>
<th>Ips lactea</th>
<th>Ips mexicana</th>
<th>Hylurgops porus</th>
<th>Hylastes longicollis</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>−</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Myrcene</td>
<td>−</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>−</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3-Carene</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>β-Phellandrene</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Primary attraction to high-girdled lodgepole pine has been demonstrated for *H. longicollis* (misidentified as *H. gracilis*) (Miller et al. 1986). Harrington (1983) found that *H. longicollis* and *H. porus* were preferentially attracted to stumps and wounded stems of ponderosa pine. The response of these species to monoterpenes, particularly 3-carene (Table 1), could provide the basis for primary attraction. Furthermore, our results strongly suggest that responses to kairomones can be species-specific. Of four species examined, specific preferences were found in all four on the basis of monoterpenes alone (Table 2). Pheromones need not be the sole constituent of species-specific chemical messages.

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References


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