Naturwissenschaften 77, 385 – 387 (1990)

Pheromone Production in a Bark Beetle Independent of Myrcene Precursor in Host Pine Species

J. A. Byers
Department of Ecology, Animal Ecology, Lund University, S-223 62 Lund

G. Birgersson
Department of Chemical Ecology, Göteborg University, S-400 33 Göteborg

The California five-spined engraver, *Ips paraconfusus* (Coleoptera: Scolytidae), is an important pest of young pine forests in California and Oregon, where nearly all pine species within the range of this bark beetle are attacked [1]. The aggregation pheromone produced by males has been identified as a synergistic blend of three components (*S*)-(−)-ipsenol, (*S*)-(+)-ipsdienol, and (4*S*)-cis-verbenol [2]. Ipsenol and ipsdienol accumulate only in males exposed to vapors of the host tree monoterpene, myrcene, in a logarithmic relationship [3]. Recently emerged control males and females contain no volatile terpene compounds, neither pheromone components nor myrcene, while only myrcene is present in vapor-exposed females [3]. Hendry et al. [4] used deuterium-labeled myrcene to prove that myrcene could be converted by hydroxylation to ipsenol and ipsdienol in males exposed to vapors. Another host tree monoterpene, (−)-α-pinene, is converted in the vapor phase to cis-verbenol in both sexes [5, 6].

A paradigm has been established that *I. paraconfusus*, and probably most other bark beetles of the genus *Ips*, use myrcene and α-pinene in their host tree as precursors to the aggregation pheromone components, ipsenol/ipsdienol and cis-verbenol, respectively [3 – 8]. Host selection and host suitability may then depend, in part, on the concentration of myrcene and α-pinene in the tree [9]. Pine trees exhibit a wide variation within and between species in their composition of myrcene and α-pinene, among other monoterpenes [10, 11]. Thus, it has also been hypothesized that tree genotypes could have evolved to have lower titers of aggregation pheromone precursors as part of a resistance mechanism to bark beetles [8].

We compared the relative attractiveness of five pine host species, ponderosa (*Pinus ponderosa*), sugar (*P. lambertiana*), Jeffrey (*P. jeffreyi*), digger (*P. sabiniana*), and lodgepole (*P. contorta*), that were infested with *I. paraconfusus* in order to detect possible differences in pheromone release and host suitability. Beetles were reared from naturally infested ponderosa pine collected from the Sierra National Forest, California. Fifty males were introduced (18:00 Aug. 29, 1985) to holes drilled in logs of each of the five host pines cut 2 days previously. These logs were wrapped with window screen and placed in sticky traps. A trap consisted of 6-mm mesh screen cylinders (19 cm, diam., 30.5 cm high) coated with Stikem Special® at 1.2 m height. Traps were separated 10 m apart in a line (Sierra National Forest, California).

Collections of flying beetles at each of the five infested logs were similar except for an approximate doubling of catch on the Jeffrey pine log (Fig. 1A). In all cases, attraction rates were significant since one to two or more females could have joined a male in his nuptial chamber if not intercepted by the traps, a natural pairing ratio in *I. paraconfusus* [12]. Another measure of the strength of a pheromone signal is the sex ratio of catch; a higher female-biased ratio in *I. paraconfusus* indicates a higher release of pheromone, because high release rates cause inhibition of male response [12]. The collected sex ratios were similar to previous reports [12], though the sex ratio on Jeffrey pine (♂ : ♀ = 1 : 15.6) was significantly higher than on the other pine species (Fig. 1A), again indicating that a somewhat more potent attractant was released. Five days after introducing the males, the logs were dissected and no significant differences were noted in survival or in general appearance of nuptical chambers (43 – 47 per log).

To determine the quantities of the pheromone components ipsenol, ipsdienol, and cis-verbenol in the feeding males, they were removed from nuptial chambers and their hindguts extracted in groups of eight in 150 μl diethyl ether with 10 ng heptyl acetate per μl as an internal quantification standard. Monoterpenes, including myrcene and α-pinene, were extracted similarly from three samples of uninfested phloem (15 – 25 mg dry weight) from each of the infested logs using 250 μl ether per sample, as well as from oleoresin (Table 1). Pheromone components and ipsenone in the males, and monoterpene hydrocarbons from the host were quantified by gas chromatography and mass spectrometry (GC-MS) using fused silica capillary columns (Fig. 1) and interpreted with respect to synthetic chemical standards (from Borregaard, Sarpsborg, Norway, and Aldrich Chemical Co., Milwaukee,

Table 1. Amounts of α-pinene and myrcene in phloem samples (g dry weight, $n = 3$) from five species of pine fed on by *Ips paraconfusus* (Sept. 3, 1985) and in oleoresin (Oct. 17, 1986). The major monoterpene in ponderosa and Jeffrey oleoresin was 3-carene (498.5 and 16.4 μg/μl, respectively), while α-pinene predominated in digger, and lodgepole resin was mostly limonene (723 μg/μl). Total monoterpene percentage (including α- and β-pinene, myrcene, 3-carene and limonene) of oleoresin was 83.5% for ponderosa, 89.9% for lodgepole, 2.9% for Jeffrey and 0.08% for digger, consistent with n-heptane as the major constituent of oleoresin in the latter two species [11]

Pine species	Amount [μg per g phloem or μl resin]	
	α-Pinene	Myrcene
Ponderosa		
phloem	<1.3–16.4	1.6–6.6
oleoresin[a]	34.9–48.8	94.9–139.0
Sugar phloem	3.8–7.9	2.4–2.6
Jeffrey		
phloem	152–445	15.8–52.6
oleoresin	1.32	3.37
Digger phloem	<0.01	<0.01
oleoresin	0.68	<0.01
Lodgepole		
phloem	35.0–76.3	18.6–39.0
oleoresin	43.2	23.7

[a]Four samples from cardinal directions of one tree

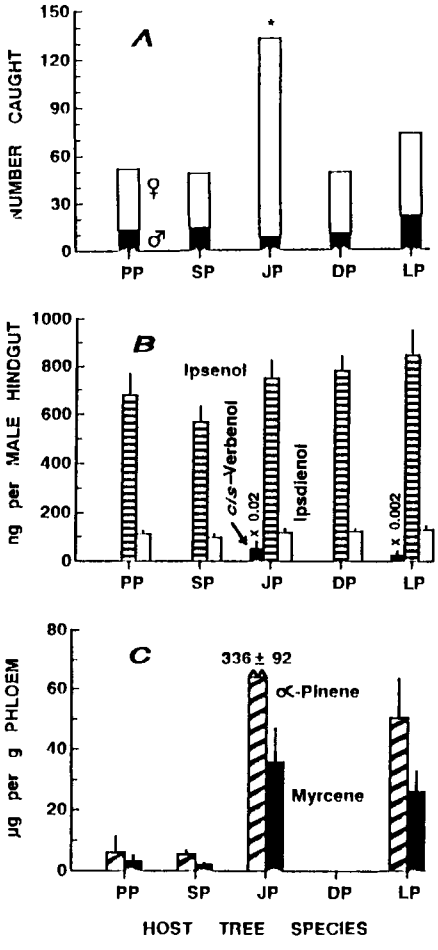

Fig. 1. A) Catch of *Ips paraconfusus* attracted to logs (28 × 15 cm diam.) of ponderosa pine (*PP*), sugar pine (*SP*), Jeffrey pine (*JP*), digger pine (*DP*), and lodgepole pine (*LP*) each infested with 50 male *I. paraconfusus* (Aug. 31 – Sept. 2, 1985). The catch of Jeffrey pine (*asterisk*) was significantly more than on either PP, SP, or DP, but not LP (arcsin transformations, *t*-tests, $P < 0.01$, $n = 4$). B) Amounts of pheromone components ipsenol, ipsdienol, and (4*S*)-cis-verbenol in the hindguts of male *Ips paraconfusus* after feeding in either PP, SP, JP, DP, or LP (above) for 5 days (Aug. 29 – Sept. 3, 1985). Quantification was by GC-MS on a Finnigan model 4021 using a column of fused silica (0.15 mm i.d. × 25 m) coated with Superox® FA (Alltech, TPA-treated PEG, df = 0.3 μm) on a temperature program of 50 °C for 4 min, rising to 200 °C at 8 °C/min and isothermal for 10 min and helium carrier gas at 25 cm/s. Limit of detection was 0.03 ng/male. C) Amounts of α-pinene and myrcene in phloem (dry weight) from logs fed on by *Ips paraconfusus* males (Sept. 3, 1985). Phloem extracts were analyzed by GC using a fused silica column (0.2 mm i.d. × 12.5 m) coated with SE®-54 CL (General Electric, 1% vinyl-, 5% phenyl-, 94% methylpolysiloxane) on a temperature program of 60 °C for 3 min, rising to 220 °C at 5 °C/min and isothermal for 15 min. Nitrogen, 20 cm/s, was used as carrier gas. Identities of the monoterpene hydrocarbons were confirmed using GC-MS as in (B) above. Vertical lines atop bars (B, $n = 5$; C, $n = 3$) represent the SEM

Wisconsin). Ipsenone was prepared by oxidation of ipsenol in Jones reagent [13].

The hindguts contained only a few major components, with ipsenol and ipsdienol dominating. Ipsenone, the ketone corresponding to ipsenol, was observed for the first time in *I. paraconfusus*, in males feeding in all pine species at 112 ± 30 (ng/male ± SD, $n = 5$). Ipsenone, rather than ipsdienone which has not been found, may account for the observed loss of deuterium from C(4) during the conversion of ipsdienol to ipsenol [7]. The generally similar attraction to each of the pine species agrees with the similar amounts of the pheromone components, ipsenol and ipsdienol, found in the hindguts of the feeding males (Fig. 1B). The increased catch on Jeffrey, and to a lesser extent on lodgepole, can be explained by the higher amounts of cis-verbenol, the third pheromone component (Fig. 1B). Although cis-verbenol was undetected by GC-MS (< 0.5 ng/male) in males feeding in the other pines, it was presumably released in sufficient quantities to be synergistically active with ipsenol and ipsdienol [2].

Unless enzyme saturation occurs even at the lowest concentrations of precursor found in nature, the paradigm predicts there should be a positive relationship between amounts of precursors in the host and pheromone products in the beetle. There appears to be a positive correspondence between higher levels of α-pinene in Jeffrey and lodgepole pines and levels of cis-verbenol in males from these species (Figs. 1B, C). However, there was no significant relationship between the widely varying amounts of myrcene in the host pines and the uniform amounts of ipsenol and ipsdienol in the males (slopes were not different from 0, *t*-test; Figs. 1B, C). The absence of myrcene in digger pine is in agreement with earlier reports [11] that show the oleoresin contains 95% n-heptane, and no mention of monoterpenes. There was a moderate variation within a pine species of α-pinene and myrcene that was not as large as between species (ranges in Table 1) and was probably due to uneven distributions of resin pockets among the rather small sample units (15–25 mg dry weight). However, the sample unit was equivalent to about 80% of a beetle's "nuptial chamber" and thus indicates that individuals in the same or different trees could ingest large differences in monoterpene hydrocarbons such as myrcene [6].

The question arises whether there is enough myrcene in the beetle's diet (host phloem) or in oleoresin to account for the quantities of ipsenol and ipsdienol found in the hindguts. The vapor concentration of myrcene in a nuptial chamber of ponderosa pine (2.8

$\times 10^{-8}$ g/ml) is expected to account for only 2% at most of the ipsenol in feeding males [6]. The absence of myrcene in digger pine phloem makes it unlikely that this compound could serve as a precursor. Allowing that a trace of myrcene was present in the oleoresin of digger pine (at most 0.01 $\mu g/\mu l$, our quantification limit), and assuming very conservatively that this was converted completely to the components and no components were released, a beetle would need to eat at least 80 μl or eight times its weight in oleoresin, and even higher amounts to account for releases of pheromone during the feeding period. This appears to be virtually impossible since the males' guts were observed to contain phloem, and oleoresin is toxic to bark beetles (*I. paraconfusus* and *Dendroctonus brevicomis*) [6, 14].

From our results it is apparent that all five pine species are about equally suitable as hosts, at least in terms of adult survival, nuptial chamber construction, pheromone production, and attraction. The production of similar amounts of ipsenol and ipsdienol in all pine species regardless of myrcene titer, as well as the inadequate supply of myrcene in digger pine, indicate that males may utilize precursors other than myrcene predominantly. This implies that insect-plant coevolution of host tree selection and resistance cannot be affected by variation of myrcene in the tree.

Received April 6 and May 10, 1990

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