E-myrcenol in *Ips duplicatus*: An aggregation pheromone component new for bark beetles

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Summary. Males of the Eurasian bark beetle *Ips duplicatus*, when feeding in host Norway spruce (*Picea abies* (L.) Karst.), produced and released ipsdienol and *E*-myrcenol, which we show to be aggregation pheromone components. Bioassays using walking beetles indicated that *E*-myrcenol in synergistic combination with ipsdienol is essential for attraction. Synergism of *E*-myrcenol and ipsdienol released at natural rates in the forest was also demonstrated with a new technique using mechanical slow-rotation of sticky traps.
Key words. Pheromone; *E*-myrcenol; ipsdienol; *Ips duplicatus*; Coleoptera; Scolytidae; *Picea abies.*

The genera *Ips* and *Dendroctonus* include most of the 'aggressive' tree-killing bark beetles that account for the major losses of coniferous trees in the northern hemisphere[1,2]. These species release pheromones, leading to the aggregation of the beetles on a tree and the overpowering of its resinous defenses[1,2]. In the genus *Ips*, no aggregation pheromone components with a monoterpene structure have been discovered since ipsenol, ipsdienol and *cis*-verbenol were identified in 1966 in the American bark beetle *I. paraconfusus*[3]. Most *Ips* species use these semiochemicals alone or in mixtures as pheromone components[1-4]. A few additional compounds have been suggested as aggregation pheromone components, among which only 2-methyl-3-buten-2-ol (methylbutenol) in European *I. typographus* has been confirmed as significantly active[2,5,7].

Ipsdienol is produced by males of *I. duplicatus* feeding in spruce logs and is attractive alone[6]. The ipsdienol found in males consists of an equal ratio of (+)- and (−)-enantiomers (Birgersson, unpublished). Commercial baits for *I. typographus* consisting of ipsdienol, *cis*-verbenol and methylbutenol are also attractive to *I. duplicatus*[7], but it is not known whether the latter two compounds are essential. Therefore, in order to determine whether ipsdienol alone is responsible for aggregation, the attractiveness of a range of release rates of racemic ipsdienol was compared in a laboratory bioassay to that of volatiles from males feeding in a host log. Females were tested for their upwind attraction to an odor source as they walked in a 42-cm diameter arena[8]. In the bioassay, release rates spanning five orders of magnitude, from 0.02 to 2000 ng ipsdienol per min., were of low attractiveness (< 23% response) with the 20 ng/min. rate being most attractive (table). The attraction of females to the infested log was much higher (75%), indicating that additional components participate in eliciting the natural attraction (table).

To identify potential pheromone components in *I. duplicatus*, males were collected from nuptial chambers in a tree during the first days of attack (Torsby, Värmland, Sweden, in May 1982). Males were stored in liquid nitrogen until extraction of their hindguts in pentane with an internal standard of heptyl acetate, as described earlier[9]. Volatiles in the extracts were identified and quantified by gas chromatography and mass spectrometry (GC-MS) (fig. 1). Besides ipsdienol, other formerly discovered

Attraction of female *Ips duplicatus* in the laboratory walking bioassay to odors from a Norway spruce long infested with males and to blends of synthetic pheromone candidates each released at 20 ng/min. in diethyl ether

Stimulus	% Females responding[b]	N
Air blank	6.7[b]	30
30-male log[a]	75.0[a]	60
Ipsdienol	22.5[b]	40
Blend = (*E*-myrcenol + ipsdienol + *cis*-verbenol + methylbutenol)	62.9[a]	70
Blend without *E*-myrcenol	23.3[b]	30
Blend without ipsdienol	6.7[b]	30
Blend without methylbutenol	60.0[a]	30
Blend without *cis*-verbenol	66.7[a]	30

[a] Males were introduced to a 25 cm × 11 cm diameter log for 40 h before placement in a 5-l bottle for 8 h, then air was purged through the bottle at 300 ml/min for 1 h prior to and during bioassays. [b] Values followed by the same letter were not significantly different ($\alpha = 0.05$, χ^2).

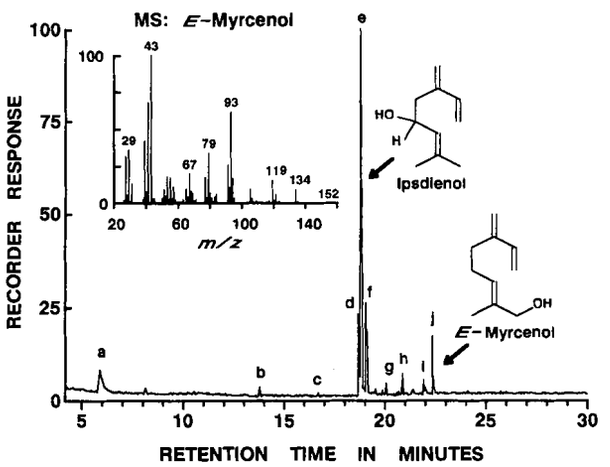

Figure 1. Gas chromatogram of volatiles in an extract of 31 male *Ips duplicatus* hindguts after collection of the beetles during their attack on a Norway spruce tree (Värmland, Sweden, May 1982). The following compounds were quantified (per male) corresponding to the letters above: a = 18 ng 2-methyl-3-buten-2-ol; b = heptyl acetate (internal standard); c = 0.3 ng ipsenol; d = 16 ng *cis*-verbenol; e = 55 ng ipsdienol; f = 18 ng *trans*-verbenol; g = 0.8 ng verbenone; h = 3.9 ng myrtenol; i = 0.8 ng 2-phenylethanol; j = 12 ng *E*-myrcenol. A Finnigan 4021 GC-MS was used with a fused silica capillary column (25 m long × 0.15 mm i.d.) coated with Superox® FA (Alltech, terephthalic acid treated polyethyleneglycol, df = 0.3 µm) on a temperature program of 50 °C for 4 min, 8°/min to 200 °C and isothermal for 10 min (He carrier gas at 25 cm/s). The 70 eV mass spectrum of *E*-myrcenol is shown in upper left graph.

pheromone components for the genus *Ips* were identified; these were methylbutenol and *cis*-verbenol which, as mentioned above, have been included in commercial baits for *I. typographus* that are attractive. However, subtraction of each of these compounds from a blend containing ipsdienol, *E*-myrcenol, methylbutenol and *cis*-verbenol indicated that neither of the latter two volatiles was a synergistic compound and that ipsdienol and *E*-myrcenol are essential pheromone components for *I. duplicatus* (table).

I. duplicatus has been placed in a taxonomic group with *I. pini, I. avulus, I. oregonis*, and *I. bonanseai*[12]. *E*-myrcenol has been found in *I. schmutzenhoferi* and *I. sexdentatus* but it is not known whether the compound has any behavioral effects[13]. In American *I. pini, E*-myrcenol is also produced by feeding males[14], but in this species it is reported to inhibit the attractiveness of ipsdienol (which is attractive alone) over a wide range of concentrations[15].

E-myrcenol (2-methyl-6-methylene-*E*-2,7-octadien-1-ol) was identified by GC-MS as a major hindgut constituent in *I. duplicatus* (fig. 1) by comparison with a synthetic standard obtained from redistilled commercially available myrcene (Aldrich) by selendioxide oxidation[10]. Neither ipsdienol nor *E*-myrcenol were found in unfed males from our laboratory colony. When *E*-myrcenol was subtracted from the chemical blend, the attraction of females was significantly reduced from 63% to about 23%, a level similar to the attractiveness of ipsdienol alone (table). The attraction to the blend without ipsdienol (7%) was not significantly different from the blank, indicating that both ipsdienol and *E*-myrcenol are synergistic aggregation pheromone components. Analysis of volatiles collected from air surrounding a male-infested log[7] revealed that ipsdienol and *E*-myrcenol were released at about 4.2 and 0.2 ng/male/min, respectively.

The synergistic effect of *E*-myrcenol together with ipsdienol on attraction of *I. duplicatus* was also demonstrated in the field by comparison of catches on a pair of sticky traps, one releasing the two-component blend and the other ipsdienol alone. The two 30 cm × 30 cm diameter tubular screen traps were coated with adhesive (Stikem special®) and separated 6 m apart horizontally at a height of 1.5 m on a metal pole that was slowly rotated by a gear motor at two revolutions per hour. With this method each trap is exposed to all possible paired positions during the trapping period, which minimizes the variation in catch that would otherwise occur with only a few fixed trap positions (traditionally the largest component of variation[11]). Although the flying population level of *I. duplicatus* was low, the attraction to the two-component blend in the rotating trap pair was significantly higher than to ipsdienol alone (Wilcoxon match pair test, $n = 18$, $p < 0.01$; or comparison with 50:50 ratio, null hypothesis, Chi square $p < 0.001$, fig. 2).

Analysis by this more powerful Chi square test is appropriate since it is expected that population variation about the paired traps would be homogenized by the trap rotation method. A check of the method is reflected in the catch of *I. typographus*, which was about equally distributed between the trap pairs, as might be expected if *E*-myrcenol were unattractive and beetles were simply intercepted in flight by the slowly rotating traps (fig. 2). Slow-rotation trap tests with the known pheromone components of *I. typographus*, methylbutenol and *cis*-verbenol, when compared with a blank, showed a 30:1 ratio of catches between the trap pair (Byers, unpublished). We believe our method of slowly rotating traps, which smooths out the variation in densities of flying

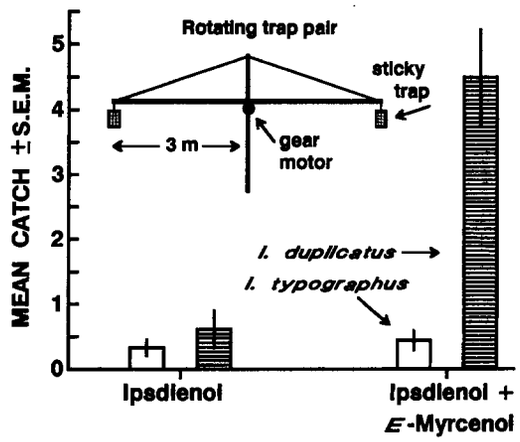

Figure 2. Catch of *Ips duplicatus* and *I. typographus* on a pair of slowly rotating sticky traps (30 cm × 30 cm diameter) baited with racemic ipsdienol and ipsdienol plus *E*-myrcenol (n = 7, Värmland, Sweden, 23 May – 16 June 1989; n = 11, Ås, Norway, 6–13 June 1990). Release rates for ipsdienol and *E*-myrcenol were each about 100–200 ng/min (equivalent to natural rates from at least 50 males). The sex ratio of *I. duplicatus* caught on the most attractive bait was 1.5 females per male (1.0–2.4, 95% binomial confidence interval).

insects with respect to trap position, will allow more reliable discrimination among behaviours elicited by semiochemical blends than current methods permit.

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