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FIELD RESPONSE OF SPRUCE BARK BEETLE, *Ips typographus*,[1] TO AGGREGATION PHEROMONE CANDIDATES[2]

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Abstract—Six compounds previously identified from hindguts of unmated male *Ips typographus* (L.) during host colonization: 2-methyl-3-buten-2-ol (MB), *cis*-verbenol (cV), *trans*-verbenol (tV), myrtenol (Mt), *trans*-myrtanol (tM), and 2-phenylethanol (PE), were tested for their attractivity in the field with a subtractive method. The amounts of MB and cV released from a pipe trap were similar to those given off from the commercial bait Ipslure as well as that from a Norway spruce tree, *Picea abies* (L.) Karst., under mass attack. The blend of the compounds became nonattractive when either MB or cV was subtracted, while subtraction of any of the other four compounds had no effect. Addition of ipsdienol (Id) to the blend did not significantly increase the attraction. In a second comparative test, the addition of three compounds as a group (tV + Mt + PE) to MB + cV again had no effect on the attraction, but the addition of Id increased the catch somewhat. Addition of host logs to a bait releasing MB + cV at a rate lower than in previous experiments did not influence the attraction to pipe traps. Sticky traps containing natural pheromone sources (50 males in a log), which released 1–5 mg/day of MB as determined by aerations with deuterated MB as internal standard, were less attractive than a synthetic source releasing similar amounts of MB.

Key Words—2-Methyl-3-buten-2-ol, *cis*-verbenol, *trans*-verbenol, myrtenol, *trans*-myrtanol, 2-phenylethanol, ipsdienol, subtractive assay, *Ips typographus*, Coleoptera, Scolytidae, *Picea abies*, host volatiles.

[1] Coleoptera: Scolytidae.

[2] This study was made within the Swedish project "Odour Signals for Control of Pest Insects."

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INTRODUCTION

The aggregation pheromone system by which the spruce bark beetle of Eurasia, *Ips typographus* (L.), colonizes a patch, such as a tree or a part of a tree, consists of two major signals. One is an attractive signal initiating the attack, and a second is a density-regulating signal that also functions in terminating the attraction (Schlyter et al., 1986). The chemical nature of the first attractive signal is the subject of this study.

The existence of a male-produced aggregation pheromone in the spruce bark beetle has been shown in the field by Bakke (1970) and Rudinsky et al. (1971). In the laboratory, it was later shown that the pheromone was released soon after host contact, with maximum attraction reached after 4–6 hr, followed by a rapid decline after the admission of females (Schlyter and Löfqvist, 1986).

Chemical analysis by gas chromatography of *I. typographus* males indicated the presence of *cis*-verbenol (cV), *trans*-verbenol (tV), ipsenol (Ie), and ipsdienol (Id), which were also found in other *Ips* species (Vité et al., 1972). However, different combinations of these substances had rather weak attractivity in the field (Bakke, 1976). The identification of 2-methyl-3-buten-2-ol (MB) with gas chromatography–mass spectrometry (GC-MS) by Bakke et al. (1977), together with the determination of (−)-(4*S*) as the active isomer of cV (Krawielitzki et al., 1977) formed the basis for the design of lures competitive with natural pheromone sources. Ipslure® (Bakke and Riege, 1982) and Typolure II (Sauerwein and Vité, 1978) both contain MB, cV, and Id, but in different ratios, while Typolure I (Vaupel et al., 1981) and Pheroprax® (Adlung, 1979) contain only MB + cV. However, rigorous tests to determine the attractivity of the individual components MB, cV, and Id are still lacking. These lures have also been tested with α-pinene and host volatiles (resin) to increase attraction, but with less clear results (Vaupel et al., 1981; Bombosch et al., 1982).

A detailed chemical analysis of beetles in different attack phases has showed that unpaired males with a completed nuptial chamber (phase 3) had a blend of at least six major components in their hindguts (Birgersson et al., 1984): MB, cV, tV, myrtenol (Mt), *trans*-myrtanol (tM), and 2-phenylethanol (PE). A seventh compound, verbenone, was only found in trace amounts. After mating and the beginning of egg-laying, some males also had Id and Ie, while the seven other components had decreased. It is possible that all of these natural compounds of related chemical structure have behavioral activity.

In this field study we have analyzed the attractivity of the components of the natural blend by a subtractive assay (Byers et al., 1985), compared the natural blend with three lures, tested synergism between host and synthetic pheromone, and compared natural and synthetic pheromone sources.

METHODS AND MATERIALS

Substances and Release Rates

The substances tested were those of the natural blend identified from male hindguts by Birgersson et al. (1984); see Table 1 for sources, purity, and release rates. Attempts were made to adjust the release rates to represent the ratios found in male hindguts. For comparison, the absolute and relative release rates of cV and MB were measured from commercial Ipslure® dispensers.

An airflow of 100 ml/min was passed over 0.1-m sections of the laminated Borregaard/Hercon® Ipslure bands in a cuvette (diameter 5 cm), and the resulting volatiles were trapped on a plug of 325 mg Porapak®-Q. The adsorbed volatiles were extracted with 2 ml pentane, and an internal standard (100 g C_7Ac) was added. The extracts were then subjected to GC for quantification and GC–MS for identification, conditions as in Birgersson et al. (1984). The release rates were calculated to be 50, 1, and 0.3 mg/day of MB, cV, and Id, respectively, for a 1-m strip (standard length) of Ipslure dispenser after one week of aging.

Field Tests

Subtractive Assay. Dispensers with test chemicals were placed in the lower part of black drainpipe traps with white exterior funnels ("N79 with funnel" type, Regnander and Solbreck, 1981). Traps were placed about 50 m apart on clear-cuts and randomized after each replicate period which lasted a few hours to more than two days, depending on flight activity. The test was carried out at two different sites in 1982: site A (Aborrtjärnsberget), a fresh clear-cut with a very high local beetle density (> 100 trees killed the previous year), and site L (Lilltjärnsberget), an old clear-cut with a low population density (no trees killed within 1 km the previous year), close to Torsby, province of Värmland, middle Sweden, 1982.

Comparative Study. Four different clear-cut areas in 1982 near Torsby were used for the comparison between the "phase-3" (natural) and the three "commercial" compositions. Two were fresh clear-cuts with high beetle densities: site A (as above) and site T (Torkbäcken, > 10 trees killed the previous year), and two were old with lower densities: site B (Boseberget) and site G (Gäddtjärnsberget). Both sites B and G had no trees killed the previous year. The later site had an elevation difference of about 50 m, while the other had less than 20 m. Traps and positioning were as in the subtractive assay.

Host Synergism. Pipe traps without funnel ("N79" type, Bakke et al., 1983) were baited with screened logs (6 cm diameter, 30 cm long, with ten 2- to 3-cm axe cuts) freshly cut from a Norway spruce (*Picea abies* (L.) Karst.) with or without a "medium" dose of MB + cV (Table 1). Together with traps

TABLE 1. CHEMICALS, RELEASE RATES AND DISPENSERS USED IN FIELD TESTS OF AGGREGATION PHEROMONE COMPONENT CANDIDATES IN *Ips typographus*

Compound	Chemical purity (%)[a]	Source	Measured[b] release rate (mg/day) (±95% C.I.)[b]	Dispensers[c]
Subtractive and comparative tests, Värmland, Sweden, 1982				
2-Methyl-3-buten-2-ol (MB)	97	Aldrich	57.0 ± 0.8	hard vial, 1-mm hole
(4*S*)-*cis*-Verbenol (cV)	96	Borregaard	1.0 ± 0.05	hard vial, 9-mm hole
(4*S*)-*trans*-Verbenol (tV)	98	KTH	0.27 ± 0.004	"730" with 4.2-mm hole
(1*S*)-Myrtenol (Mt)	92	Aldrich	0.084 ± 0.01	"730" with 200-μl capillary
(1*S*)-*trans*-Myrtanol (tM)	>99	Fluka	0.037 ± 0.003	"730" with 150-μl capillary

2-Phenyl-ethanol (PE)	99	Kebo	0.17 ± 0.005	"730" with 150-μl capillary
Ipsdienol (Id)	95	Borregaard	0.34 ± 0.02	"730" with 2.9-mm hole
			0.21 ± 0.02	"730" with 150-μl capillary
Host synergism test, Lardal, Norway, 1983				
2-Methyl-3-buten-2-ol (MB)	97	Aldrich	5.8 ± 0.3	"730" with 50-μl capillary
(4*S*)-*cis*-Verbenol (cV)	99	Borregaard/KTH	0.05 ± 0.001	"730" with 150-μl capillary
Comparison of natural and synthetic pheromone, Gribskov, Denmark, 1984				
2-Methyl-3-buten-2-ol (MB)	97	Aldrich	5.8 ± 0.3	"730" with 50-μl capillary
			0.5 ± 0.05	"730" closed
			0.2 ± 0.02	0.25-mm-diam capillary
(4*S*)-*cis*-Verbenol (cV)	99	Borregaard/KTH	0.05 ± 0.001	"730" with 150-μl capillary
			0.01 ± 0.001	"7570," 1.1 mg of cV
			0.002 ± 0.001	"7560," 0.5 mg of cV

[a] Chemical purity estimated by capillary GC. Optical purity for cV > 94% (−)-(4*S*), Id was racemic.

[b] The release rates from polyethylene vials were estimated in the laboratory by measuring the weight loss of the vials, placed in a wind tunnel at 0.7 m/sec and 20°C, during a month's period. The rate of release was calculated as the slope, with its 95% confidence interval, from the regression of weight on time.

[c] Dispensers were polyethylene vials (Kartell, Italy) of two types: "730," a 1-ml vial of soft polyethylene; and "hard," a 2-ml vial of hard polyethylene, with capillaries or drilled holes in their lids. For the two lowest rates of cV we used polyethylene tubes, 3 cm long, heat sealed in both ends, with 0.40 mm (7570) or 0.55 mm (7560) wall thickness.

with only MB + cV, these formed an equilateral triangle, a trap group, with 10 m to a side, randomized within the group after each replicate. A total of four groups were used in an old clear-cut, north of Skien, Lardal, southern Norway in May–June 1983.

Comparison of Natural and Synthetic Pheromone. Logs (diameter 12 cm, 30 cm long) infested with 50 males or 30-cm sections of pipe traps with dispensers for synthetic pheromone (Table 1) were enclosed by a sticky trap (hardware cloth No. 4 coated with Stickem Special, Byers and Wood, 1980). Logs and pipe-trap sections were both screened with brass net (No. 60) to exclude arriving beetles and to give a similar appearance. The logs were cut from three Norway spruce [*Picea abies* (L.) Karst.] trees that represented different stages of vigor and resin content (Table 4). The trees were all cut in the evening on May 17, 1984. Male beetles were introduced, after sex separation (Schlyter and Cederholm, 1981), the day after. The males (50/log) were introduced into predrilled holes on the evening before May 18, but had a free choice to start excavating the nuptial chamber or to leave, in which case they were replaced after a few hours. Both beetles from our laboratory stock (from Lardal, southern Norway) and wild, Danish beetles (caught in pipe traps baited with synthetic pheromone in the area the days before) were used (Table 4). In all cases, each set had one log from each of the three trees infested with 50 males. The traps in a set were placed in two lines with a minimum of 10 m between traps. The two trap sets were at least at 25 m away from each other and other pheromone sources such as trees under attack. Trap positions were randomized after each replicate. Trapping sites were in clear-cuts, cleared of logging residues, near groups of trees killed the previous year in Esrum forest district, Grib Skov, north of Hillerød, Denmark, 1984.

Aerations of Natural and Synthetic Pheromone Sources. Male-infested logs and sections of pipe traps (containing dispensers with synthetic compounds) were aerated after each day of trapping. Logs and pipe-trap sections were placed individually in glass jars (ID 15 cm, 30 cm high) through which 150 ml air/min (charcoal filtered, ID 2 cm × 20 cm) was passed with a pneumatic suction ejector (AGA). The volatiles in the effluent were trapped on Porapak-Q (mesh 80–100, 170 mg). As internal standards, trideuterated-MB and α-isophorone were evaporated from fused silica capillaries (ID 0.20 mm), one end sealed with beewax, attached to the side of logs or pipe-trap sections. After 3 hr of aeration, the Porapak-Q plugs were extracted with 2 ml of diethyl ether, 500 ng C_7Ac was added as a standard for the concentration, and the extracts stored at −20°C. The extracts were concentrated and subsequently analyzed by GC−MS; conditions as in Birgersson et al. (1984).

Sample Handling and Statistics

Sample Handling. Beetles were preserved in ethanol and identified to sex by pronotal bristle density (Schlyter and Cederholm, 1981). In the subtractive

assay, at least 260 beetles (or the total catch if less than 260) from each site and bait were identified to sex. Beetles from the comparative study were not identified to sex, and, for samples of >100 individuals, their numbers were estimated by measuring the volume of *I. typographus* (42 beetles/ml).

Statistics. Raw catch data (y) were subjected to a series of transformations y^{-1}, $y^{0.5}$, log (y + constant), p^{-1} to make them suitable for ANOVA by achieving homogeneous variances (Cochran's C and Barlett box tests, $P > 5\%$) and approximately normal distributions. The transformation log (y + constant) was chosen for the first two tests as it made the data homoscedastic and gave high F ratios in ANOVA. The high number of zero catches in the two later tests caused significant heteroscedacity to remain after transformations, which made it more appropriate to perform a nonparametric test [Wilcoxon matched-pairs with significance levels adjusted for number of comparisons (Kirk, 1969)] of the untransformed data in lieu of ANOVA.

RESULTS

Subtractive Assay

Subtraction of either MB or (−)-(4*S*)-cV from the natural phase-3 blend dramatically reduced the catch of *I. typographus* (Figure 1), indicating that both MB and cV are equally essential for attraction. The relative response in the two sites was very similar, in spite of a 10-fold difference in total catch (Figure 1). Thus, (+)-(4*S*)-tV or Mt could not substitute for cV (or MB). The inclusion of tM, a component of the natural phase-3 blend, in the later replicates also did not increase the attractivity. The addition of (*R/S*)-Id to the phase-3 blend seemed to increase the catch of *I. typographus* slightly, although the difference was not statistically significant. The sex ratio was little affected by subtraction or addition of compounds. Exceptions were the removal of PE or MB, which resulted in a somewhat higher percentage of males in the catch (Table 2).

I. duplicatus Sahlb. was attracted only to the bait with Id added to the phase-3 blend. Several other scolytid species were caught in low numbers in the pipe traps with funnel, but none showed a clear pattern of attraction like the two *Ips* species. *Pityogenes chalcographus* (L.) was caught on all baits with an increase on phase 3 plus Id but the numbers were low (Table 2).

Comparison between Lures

The two baits containing Id (Ipslure, Typolure II) generally caught more beetles, although the differences were small and not statistically significant at the site with the highest number of replicates (Figure 2). The bait with the highest catches, Typolure II, had both a higher dose of Id and of cV, while Ipslure was almost identical to phase 3 at the best site. This means that it is difficult to assign a clear synergistic function of Id based on these data. When

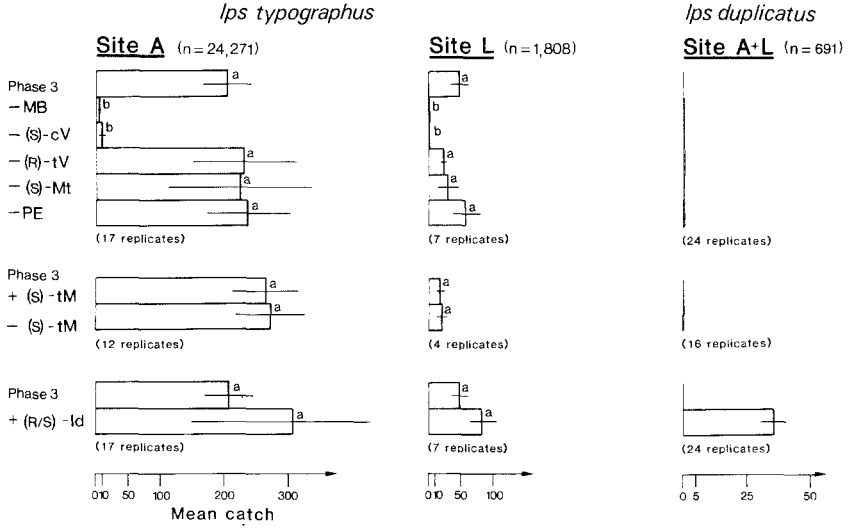

FIG. 1. Mean catches (±SE) per replicate in pipe traps with the subtractive assay of *Ips typographus* pheromone components. Subtraction or addition of a component of the phase-3 blend is indicated by + and −, respectively. Phase 3 is a bait representing the pheromone content in hindguts of unmated *I. typographus* males, which included the components MB, cV, tV, Mt, and PE during the first five replicates. For the remaining replicates, an additional trap was added which had a sixth compound, tM, which is also found in males from phase 3. One bait had the phase-3 blend plus Id, which is found in mated males. Total catch is given by (n). Release rates and names of chemicals and sex ratios of catch are shown in Table 1. Chirality of compounds with optical activity is given by *R* and *S*. Bars with the same letter are not significantly different within a site ($P > 5\%$) by ANOVA of log (catch + $\frac{1}{4}$) followed by Duncan's multiple-range test.

comparing phase 3 with Typolure I, it is evident that the addition of tV, Mt, and PE to our standard MB + cV bait (=Typolure I) did not increase the attraction, as the catches of the two baits were virtually identical in all sites (Figure 2).

Host Synergism

Volatiles from a spruce log did not enhance the attractivity of a lower, "medium" dose of MB and cV, as shown by the nearly identical mean catches (Table 3). The bait consisting of a log alone in the pipe traps without funnel did not catch a single beetle during the entire experiment, while 705 beetles were caught by the two pheromone baits. The sex ratio was not altered significantly by the addition of the log to the pheromone source. Few other scolytids were collected, probably due to the low pheromone release and the use of pipe traps without an exterior funnel.

TABLE 2. SUBTRACTIVE ASSAY OF AGGREGATION PHEROMONE CANDIDATES IN *Ips typographus*: BAITS, SEX RATIOS, AND CATCHES OF OTHER SPECIES IN PIPE TRAPS, VÄRMLAND, SWEDEN, MAY 17, TO JUNE 8

Baits (Designation)	Release rates (mg/day)[a]							Sex ratio of *Ips typographus*		Catches of other species[b] (means, site A)[b]				
										Scolytidae				*Cleridae*
	MB	cV	Id	tV	Mt	tM	PE	Male (%)	95% C.I.	Pc	Hc	Da	Tl	Thf
Phase 3 (natural composition)	57	1	—	0.3	0.1	—	0.2	20.4	17–24	0.8c	1.4	0.2	0.2	1.4
−MB	—	1	—	0.3	0.1	—	0.2	33.7	25–43	1.3c	3.3	0.5	0.1	1.0
−cV	57	—	—	0.3	0.1	—	0.2	19.4	14–26	0.8c	1.9	0.1	0.1	1.2
−tV	57	1	—	—	0.1	—	0.2	19.3	16–23	2.2c	1.4	0.3	0.3	2.8
−Mt	57	1	—	0.3	—	—	0.2	17.6	14–21	5.6de	1.8	0.4	0.2	1.6
−PE	57	1	—	0.3	0.1	—	—	31.2	27–35	1.5c	1.4	0.5	0.3	1.6
+Id	57	1	0.2	0.3	0.1	—	0.2	15.6	13–19	8.2e	1.5	0.2	0.1	2.5
+tM	57	1	—	0.3	0.1	0.04	0.2	(9.7)[c]	7–13	(3.2)[c]	2.9	0.2	<0.1	1.0

[a] For confidence intervals of release rates and abbreviations of compounds see Table 1.

[b] Pc = *Pityogenes chalcographus* (L.), Hc = *Hylastes* cf. *cunicularius* Er., Da = *Dryocoetes autographus* (Ratz.), Tl = *Trypodendron* (*Xyloterus*) *lineatum* Oliv., Thf = *Thanasimus formicarius* L. Values followed by the same letter are not significantly different by ANOVA on log (catch + 0.25) followed by Duncan's multiple-range test.

[c] tM was included in the test only during the last 12 replicates, which means that catches and sex ratios are not comparable with other baits with 17 replicates (see Figure 1).

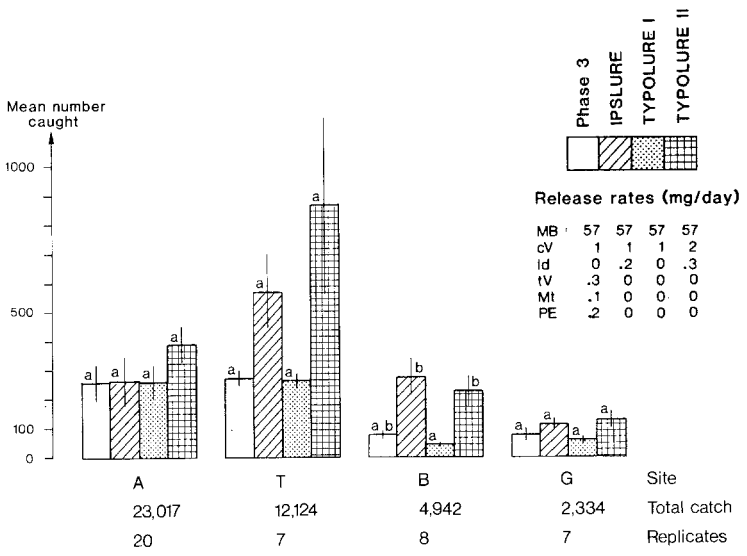

FIG. 2. Mean catches (±SE) per replicate of *I. typographus* in pipe traps with the comparative test of baits representing commercial lures and phase-3 blend (representing the natural hindgut composition). For more extensive data on release rates and names of chemicals see Table 1. Bars with the same letter are not significantly different within a site ($P > 5\%$) by ANOVA of log (catch + $\frac{1}{4}$) followed by Duncan's multiple-range test.

TABLE 3. HOST VOLATILES AND PHEROMONE SYNERGISM TEST OF *Ips typographus* WITH PIPE TRAPS, LARDAL, SOUTHERN NORWAY, JUNE 7–11, 1983, 28 REPLICATES

Baits, synthetics (mg/day)			Catch (number of beetles)[b]		Sex ratio	
MB	cV	Log[a]	Mean	Max–min	Males (%)	95% C.I.[c]
5.8	.05	No	14.2c	49–0	47.4	42–52
5.8	.05	Yes	13.5c	35–4	44.6	40–49
0	0	Yes	0.0d	—	—	—
Total catch			705			

[a]Tree cut June 1, logs 6–8 cm diam. × 28 cm (with 2- to 3-cm long axe cuts, 10 per log).
[b]Values followed by the same letter are not significantly different by Wilcoxon matched-pairs signed ranks test ($P > 5\%$) for totals, males, or females.
[c]95% Binomial confidence intervals (Byers and Wood, 1980).

Natural and Synthetic Pheromone: Attraction and Release Rates

The strongest bait in this test, the "medium" MB + cV synthetic pheromone source consistently caught more beetles than did the male-infested logs (Table 4). There was no consistent difference in the catch between logs infested with laboratory-reared males originating from Norway and logs with wild males of local origin, and the data are pooled in Table 4. The sex ratio was about 40% males on most traps but, of the synthetic baits, only the catches of the two stronger baits were significantly different from an equal sex ratio. If the catches from all three traps with male-infested logs are pooled, the proportion of males (35%) is also significantly different from an equal sex ratio (95% binomial confidence interval, Byers & Wood 1980).

The release of MB could be accurately estimated by the deuterated MB internal standard and showed that the release of MB from the logs was high and close to the "medium" synthetic bait, which was the strongest in this test. The release rates estimated by weight loss and by entrainment were in good agreement for the MB in both the "medium" and "low" baits (Table 4). The standard for the monoterpene alcohols, α-isopherone, was not released in sufficient quantities to obtain an estimate of their absolute release rates. However, the ratios between the monoterpene alcohols could be measured with precision and accuracy and were used to show that verbenone, together with readily detectable amounts of cV and tV, was released from the logs at some times in approximately equal ratios to the verbenols (Table 4).

DISCUSSION

Tests of Synthetic Compounds

MB and cV could be identified as essential for the attraction of *I. typographus*, while subtraction from the phase-3 blend of tV, Mt, or PE, or addition of tM had no apparent effect on the catch. Thus, the released amounts of (+)-tV, (−)-Mt, PE, and (−)-tM could not substitute for or increase the attraction to MB and cV. We could not substantiate the report by Dickens (1981) that (+)-(4*S*)-tV could substitute for cV and was as attractive as (−)-(4*S*)-cV when tested in sleeve olfactometers. The addition of Id increased the catch somewhat, but not significantly, as was earlier indicated by Dickens (1981) and Bakke et al. (1983). Thus Id plays, at most, a minor role in the aggregation pheromone, as tested here. However, a small, but significant, increase in catch when small amounts of Id were added to MB + cV was demonstrated by Schlyter et al. (1987).

Only combinations including ipsdienol (Id) were attractive to *I. duplicatus*, in agreement with the results of Bakke (1975). The comparative test of baits

TABLE 4. RELEASE RATES OF AND ATTRACTION TO NATURAL AND SYNTHETIC PHEROMONE SOURCES OF *Ips typographus* GRIB SKOV, DENMARK, MAY 19–JUNE 5 1984, STICKY TRAPS, 12 REPLICATES

Baits (designation)	Release rate by weight loss (mg/day)		Release rates by entrainment per day				Catch (number of beetles)		Sex ratio (% males)
	MB	cV	MB (mg)[a]	cV (μg)[b]	tV (μg)	Vn (μg)	Mean	Max–min	
Medium	5.8	0.05	2.8	0.8	0	0	22.7	55–14	40[e]
Low_1	0.5	0.01	0.2	0.6	0	0	5.5	22–0	38[e]
Low_2	0.1	0.002	0.1	0.5	0	0	2.1	5–0	46
Log + 50♂♂									
Tree MI[c]	—	—	1.0	0.3	0.3	0.3	3.6[d]	12–0	44
Tree MII[c]	—	—	4.6	0.1	0.4	0.5	1.7[d]	6–0	(29)
Tree N[c,f]	—	—	1.5	0.4	0.8	0.7	1.6[d]	6–0	(20)
Control (blank)	—	—	0	0	0	0	1.7	5–0	(24)

[a] MB release estimated by GC-MS via the *m/z* 71:74 relative to the release of d_3-MB in the aeration vial. Values given for release by entrainment are means of three or more aerations of the same bait. For release by weight loss and abbreviations of compounds see Table 1.

[b] cV, tV, Vn calculated relative to the 500 μg C_7Ac added after extraction of the adsorbent.

[c] Two sets of traps were used, with five and seven replicates each, and results pooled as no difference between the sets could be detected. In one of the two sets the laboratory-beetle-infested logs were replaced with local, wild beetles caught in pheromone traps after the second replicate. No differences in relative or absolute catches could be noted between wild and laboratory beetles.

[d] Significantly different from "Medium" at $P < 5\%$ by Wilcoxon matched-pairs signed ranks test, significance level (α) corrected for number of planned comparisons (Kirk, 1969).

[e] Significantly different from 50% males (95% binomial C.I.), values within ( ) indicate less than 20 beetles caught.

[f] Tree N of low apparent vigor (crown narrow with low needle density, "transparent") with a mean annual increment for the last five years of 3.3 mm/year, while MI and MII were of high apparent vigor with dense, dark green crowns with 6.8 and 6.7 mm increments, respectively.

representing commercial lures showed that even the addition of tV, Mt, and PE as a group did not enhance the attractivity of the MB + cV combination. The addition of a small amount of Id, in combination with a doubled dose of cV (Typolure II), produced the bait with the highest catch, but significantly so in only one of four test sites. As the known attractant cV also was increased, the effects of Id might be confounded, so we cannot, in this case, confirm a benefit of Id in the attractant blend. However, the bait representing Ipslure also caused high catches, which, in one of four sites, was significantly different from one of two possible controls. One reason that none of the hindgut components other than MB and cV (and possibly Id) were found active in this study could be that the tV, Mt, tM, and PE tested had impurities of an inhibitory nature or they were not of the appropriate enantiomeric composition. However, the tV tested was the same enatiomer, (+)-(4*S*)-tV, as tested by Dickens (1981), and the (4*S*)-enantiomer is probably the one naturally produced (Klimetzek and Francke, 1980; Birgersson et al., unpublished). The natural enantiomeric composition of Mt and tM produced by the beetles is not known, and we used only one enantiomer. Thus, the possibility remains that a full, enantiomerically correct blend of phase 3 might be more active than MB + cV alone.

Host Synergism and Natural/Synthetic Comparison

Examples exist in several bark beetle genera of an aggregation pheromone synergized by volatiles from living host material: *Scolytus multistriatus* and elm logs (Peacock et al., 1984), and *Tomicus minor* and pine logs (Lanne et al., 1987), or by identified host compounds: *Dendroctonus brevicomis* and myrcene (Bedard et al., 1969), *D. ponderosae* or *D. frontalis* and α-pinene (Pitman, 1971; Renwick and Vité, 1969), and *Gnathotrichus* spp. and α-pinene (Borden et al., 1980). Tests with *I. typographus* in Germany have indicated that α-pinene or spruce resin added to traps with synthetic pheromone did not increase trap catches (Vaupel et al., 1981). However, spruce logs in one experiment increased catch considerably (Bombosch et al., 1982), although proper controls and replication appear to be lacking. Our test showed no increase in catch in pipe traps with spruce logs added. Comparisons between experiments using logs are made difficult by the fact that, as in our experiment, the release rates of the host compounds are usually not known, but only the size of logs used. In our test the logs were rather small and volatile release may have declined too soon to show possible weak pheromone synergism effects. However, the lack of any strong host attraction or synergism found in this study is not surprising, as other *Ips* species have not conclusively been shown to have a long-range host attraction behavior or synergism between pheromone and host odors (Wood, 1982).

The poor attractivity of the natural pheromone sources (50 males in log) is more surprising, especially as the chemical analysis of aerations showed that the release of MB from the logs was similar to "medium," the strongest syn-

thetic bait in this test. MB is believed to be produced de novo by the beetle in the appropriate biological phase. The high production of MB, similar to that of beetles mass-attacking trees (Birgersson, unpublished), indicates that the beetles in the test logs were in the appropriate physiological and behavioral condition for maximal pheromone production. The period of the test (18 days for one set of logs) is not unreasonably long, as walking beetles in a Y-tube bioassay were attracted to male-infested logs for 16 days (Schlyter and Löfqvist, 1986). However, a low and declining release of cV (produced from the host monoterpene α-pinene) may well explain the low attractivity of the logs. Byers (1981) showed for *I. paraconfusus* Lanier males that the pheromone components, ipsenol and ipsdienol (produced from host myrcene), began to decline after about six days and were undetectable (GLC) after two weeks. Furthermore, verbenone (Vn) decreases trap catches (Bakke, 1981), and although its release rate was low, it could also have contributed to the low catches. The release of Vn from infested logs is probably due to the activity of microorganisms in the host tissue as they convert pinene and verbenols to Vn (Brand et al., 1975; Leufvén et al., 1984), while male hindguts contain very little Vn (Birgersson et al., 1984).

If the pheromone system in *I. typographus* in fact consists of only two components (MB + cV), one might ask if such a simple system is specific enough to ensure species specificty of the pheromone signal. The monoterpene alcohol cV is found in several related and/or sympatric species (Vité et al., 1972; Wood, 1982). However, the enantiomeric ratios and behavioral roles of cV are not well characterized in these species. As yet, the very large amount of the isoprene alcohol MB in the two-component system appears unique to *I. typographus* among *Ips* species and probably plays the major role to ensure the species specificity of the pheromone.

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REFERENCES

ADLUNG, K.G. 1979. Versuchsergebnisse zur Anlockung des Buchdruckers (*Ips typographus* L.) mit Lockstoff-Dispensoren. *Allg. Forst Jagdztg.* 150:125–127.

BAKKE, A. 1970. Evidence of a population aggregating pheromone in *Ips typographus* (Coleoptera: Scolytidae). *Contrib. Boyce Thompson Inst.* 24:309-310.

BAKKE, A. 1975. Aggregation pheromone in the bark beetle, *Ips duplicatus* (Sahlberg). *Norw. J. Entomol.* 22:67-69.

BAKKE, A. 1976. Spruce bark beetle, *Ips typographus*: Pheromone production and field response to synthetic pheromones. *Naturwissenschaften* 63:92.

BAKKE, A. 1981. Inhibition of the response in *Ips typographus* to the aggregation pheromone; field evaluation of verbenone and ipsenol. *Z. Angew. Entomol.* 92:172-177.

BAKKE, A., and RIEGE, L. 1982. The pheromone of the spruce bark beetle *Ips typographus* and its potential use in the suppression of beetle population, pp. 3-15, in A.F. Kydonieus, and M. Beroza, (eds.). Insect Suppression with Controlled Release Pheromone Systems, Vol. II. CRC Press, Boca Raton, Florida.

BAKKE, A., FRØYEN, P., and SKATTEBØL, L. 1977. Field response to a new pheromonal compound isolated from *Ips typographus*. *Naturwissenschaften* 64:98.

BAKKE, A., SAETHER, T., and KVAMME, T. 1983. Mass trapping of the spruce bark beetle *Ips typographus*. Pheromone and trap technology. *Medd. Nor. Inst. Skogforsk.* 38:1-35.

BEDARD, W.D., TILDEN, P.E., WOOD, D.L., SILVERSTEIN, R.M., BROWNLEE, R.G., and RODIN J. O. 1969. Western pine beetle: Field response to its sex pheromone and a synergistic host terpene, myrcene. *Science* 164:1284-1285.

BIRGERSSON, G., SCHLYTER, F., LÖFQVIST, J., and BERGSTRÖM, G. 1984. Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases. *J. Chem. Ecol.* 10:1029-1055.

BOMBOSCH, S., JOHANN, M., and RAMISCH, G. 1982. Versuche zur Verbesserung der Fangsterbegnisse von Borkenkäferfallen. *Holz-zentralblatt* 129:1852-1853.

BORDEN, J.H., LINDGREN, B.S., and CHONG, L. 1980. Ethanol and α-pinene as synergists for the aggregation pheromones of two *Gnathotrichus* species. *Can. J. For. Res.* 10:290-292.

BRAND, J.M., BRACKE, J.W., MARKOVETZ, A.J., WOOD, D., and BROWNE, L. 1975. Production of verbenol by a bacterium isolated from bark beetles. *Nature* 254:136-137.

BYERS, J.A. 1981. Effect of mating on terminating aggregation during host colonization in the bark beetle, *Ips paraconfusus*, *J. Chem. Ecol.* 7:1135-1147.

BYERS, J.A., and WOOD D.L. 1980. Interspecific inhibition of the response of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus*, to their pheromones in the field. *J. Chem. Ecol.* 6:149-164.

BYERS, J.A., LANNE, B.S., LÖFQVIST, J., SCHLYTER, F., and BERGSTRÖM, G. 1985. Olfactory recognition of host-tree susceptibility by pine shoot beetles. *Naturwissenschaften* 72:324-326.

DICKENS, J.C. 1981. Behavioural and electrophysiological responses of the bark beetle, *Ips typographus*, to potential pheromone components. *Physiol. Entomol.* 6:251-261.

KIRK, R.E. 1969. Experimental Design: Procedures for the Behavioural Sciences. Brooks/Cole, Belmont.

KLIMETZEK, D., and FRANCKE, W. 1980. Relationship between the enantiomeric composition of α-pinene in host trees and the production of verbenols in *Ips* species. *Experientia* 36:1343-1344.

KRAWIELITZKI, S., KLIMETZEK, D., BAKKE, A., VITÉ, J.P., and MORI, K. 1977. Field and laboratory response of *Ips typographus* to optically pure pheromonal components. *Z. Angew. Entomol.* 83:300-302.

LANNE, B.S., SCHLYTER, F., BYERS, J.A., LÖFQVIST, J., LEUFVÉN, A., BERGSTRÖM, G., VAN DER PERS, J.N.C., UNELIUS, R., BAECKSTRÖM, P., and NORIN, T. 1987. Difference in attraction to compounds similarly present in two sympatric pine shoot beetles *Tomicus minor* and *T. piniperda*. *J. Chem. Ecol.* In press.

LEUFVÉN, A., BERGSTRÖM, G., and FALSEN, E. 1984. Interconversion of verbenols and verbenone by identified yeasts isolated from the spruce bark beetle *Ips typographus*. *J. Chem. Ecol.* 10:1349-1361.

PEACOCK, J.W., WRIGHT, S.L., and FORD, R.D. 1984. Elm volatiles increase attraction of *Scolytus multistratus* (Coleoptera: Scolytidae) to multilure. *Environ. Entomol.* 13:394–398.

PITMAN, G.B. 1971. *Trans*-Verbenol and alpha-pinene: Their utility in manipulation of the mountain pine beetle. *J. Econ. Entomol.* 64:426–430.

REGNANDER, J., and SOLBRECK, C. 1981. Effectiveness of different types of pheromone traps used against *Ips typographus* (L.) (Col., Scolytidae) in Sweden. *Anz. Schaedlingskde Pflanz. Umweltschutz* 54:104–108.

RENWICK, J.A.A., and VITÉ, J.P. 1969. Bark beetle attractants: Mechanism of colonization by *Dendroctonus frontalis*. *Nature* 224:1222–1223.

RUDINSKY, J.A., NOVAK, V., and SVIHRA, P. 1971. Pheromone and terpene attraction in the bark beetle *Ips typographus* L. *Experientia* 27:161–162.

SAUERWEIN, P., and VITÉ, J.P. 1978. Die Eignung von Typolur-Formulierungen zur Überwachung and Bekämpfung des Buchdrucker *Ips typographus*. *Mitt. Dtsch. Ges. Allg. Angew. Entomol.* 1:189–192.

SCHLYTER, F., and CEDERHOLM, I. 1981. Separation of the sexes of living spruce bark beetles, *Ips typographus* (L.), (Coleoptera: Scolytidae). *Z. Angew. Entomol.* 92:42–47.

SCHLYTER, F., and LÖFQVIST, J. 1986. Response of walking spruce bark beetles *Ips typographus* to natural pheromone from different attack phases. *Entomol. Exp. Appl.* 41:219–230.

SCHLYTER, F., BYERS, J.A., and LÖFQVIST, J. 1987. Attraction to pheromone sources of different quantity, quality and spacing: Density-regulation mechanisms in the bark beetle *Ips typographus*. *J. Chem. Ecol.* In press.

VAUPEL, O., DIMITRI, L., and VITÉ, J.P. 1981. Untersuchungen über den Einsatz von Lockstoffbeköderten Rohrfallen zur Bekämpfung des Buchdruckers (*Ips typographus* L.), sowie Möglichkeiten der Optimerung von Lockstoffverfahren. *Allg. Forst. Jagdztg.* 152:102–113.

VITÉ, J.P., BAKKE, A., and RENWICK, J.A.A. 1972. Pheromones in *Ips* (Coleoptera: Scolytidae): Occurrence and production. *Can. Entomol.* 104:1967–1975.

WOOD, D. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Annu. Rev. Entomol.* 27:411–446.