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DIFFERENCES IN ATTRACTION TO SEMIOCHEMICALS PRESENT IN SYMPATRIC PINE SHOOT BEETLES,[1] *Tomicus minor* AND *T. piniperda*[2]

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Abstract—The chemical ecology of host- and mate-finding in the pine shoot beetles, *Tomicus minor* and *T. piniperda*, was studied in southern Sweden. Beetles were collected in the field from defined attack phases on Scots pine. Using gas chromatography–mass spectroscopy, a number of oxygen-containing monoterpenes, e.g., 3-carene-10-ol, myrtenol, *trans*-verbenol, and verbenone, were identified from hindgut extracts of both sexes of both species. Compared to *T. minor*, *T. piniperda* contained additional compounds and in larger amounts. The amounts were highest in both species at the time when the beetles had bored into contact with the resin-producing xylem-phloem tissue. The synthesis of (1*S*,6*R*)-3-carene-10-ol by photooxidation of (+)-(1*S*,6*R*)-3-carene is described. In comparative electroantennogram (EAG) measurements on males and females of both species, the most active of the tested compounds was *trans*-verbenol. Laboratory bioassays of walking beetles showed that *T. piniperda* was attracted to uninfested pine logs. *T. minor* was more strongly attracted to pine logs infested with females than to uninfested pine logs, indicating a female-produced aggregation pheromone. Field tests confirmed that *T. piniperda* was strongly attracted to pine logs. The attraction of *T. minor* to logs was significant only when logs were combined with racemic *trans*-verbenol and (1*S*,6*R*)-3-carene-10-ol. *T. minor* was also

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attracted to a combination of these monoterpene alcohols alone. We suggest that host and mate location in *T. piniperda* is achieved by means of a kairomone composed of host monoterpenes, while *T. minor* utilizes a primitive pheromone synergized by host odors. Evolution of host colonization strategies of the two beetles are discussed.

Key Words—Host attraction, pheromone, *Pinus sylvestris*, *trans*-verbenol, 3-carene-10-ol, myrtenol, verbenone, EAG, kairomone, *Blastophagus*, *Tomicus minor*, *Tomicus piniperda*, Coleoptera, Scolytidae, pine shoot beetles.

INTRODUCTION

The lesser pine shoot beetle *Tomicus minor* (Hart.) and the larger pine shoot beetle *Tomicus piniperda* (L.) (synon. *Blastophagus* Eich., synon. *Myelophilus* Eich.) (Coleoptera, Scolytidae) are both present in the palearctic region from Europe to Siberia and Japan. The two species are sympatric and mostly colonize Scots pine, *Pinus sylvestris* (L.), in western Europe but occasionally other trees. Both species hibernate as adults and disperse in the spring as soon as the daily temperature exceeds about 12°C. They usually establish galleries in the phloem of windthrown or otherwise weakened trees. *T. minor* is usually found in the smoother bark on the branches or higher trunk while *T. piniperda* prefers the thicker bark. Females in both species initiate galleries in the bark and are followed by a male. After two to three months, the brood emerges and flies to shoots of the current year's growth where they feed and mature. As a result of this feeding, both species cause growth losses and pruning of shoots. In addition to these effects, *T. minor* carries fungi that cause blue staining of the wood (cf. Francke-Grosmann, 1952). The bionomics of these species and their silvicultural importance have been reported by Richtie (1917), Bakke (1968), Postner (1974), and Långström (1983).

Kangas et al. (1970b) found that a volatile pine fraction, containing α-terpineol, and *cis*- and *trans*-carveol, was attractive to *T. minor* beetles in laboratory bioassays. Bakke and Jordal (personal communication) found *trans*-verbenol in hindguts of *T. minor*, and their field trapping experiments indicated that this compound may act as a pheromone component.

Earlier studies on the semiochemical system of *T. piniperda* have searched for aggregation pheromones (Schönherr, 1972; Carlé, 1978; Carlé et al., 1978; Francke and Heemann, 1976; Byers et al., 1985) with no apparent success, while evidence for a strong host attraction has accumulated (Kangas et al., 1965; Löyttyniemi and Hiltunen, 1976; Byers et al., 1985; Schroeder and Eidmann, 1986).

The aim of this study is to investigate the role of host- and beetle-produced odors in the host- and mate-finding of *T. minor* and *T. piniperda*.

METHODS AND MATERIALS

Collection of Beetles and GC-MS Analyses of Gut Volatiles. Beetles of both species were collected at Blentarp, 20 km southeast of Lund in the southernmost province of Sweden, from March 26 to April 15, 1982, while the beetles were dispersing and constructing galleries on Scots pine. *T. minor* were collected from several windthrown trees and large branches, while *T. piniperda* were taken from four windthrown trees (A–D). The diameter at breast height (DBH) of trees A, B, and C was approximately 30 cm and tree D was 15 cm. Tree C was cut into three sections, and the beetles were taken from the middle section. The beetles were treated and stored in liquid nitrogen as described by Birgersson et al. (1984).

Both *T. minor* and *T. piniperda* were separated according to the attack phase during their gallery construction in host trees (see Table 1). Attack phases were chosen in such a way that comparisons of the two species would be facilitated. However, an exact correspondence between phases in the two species was not possible to achieve because of their different behaviors (Table 1). Therefore, we divided the phase "pairs boring" into three subphases for *T. piniperda* according to the length of the tunnel made. Under favorable weather conditions, the time span between finding a suitable host (phase "walking") to egg-laying is three to six days.

Hindguts were dissected and extracted essentially as described by Birgersson et al. (1984). Females were also examined for presence of sperm (filled spermatheca) or eggs. Pentane extracts obtained from 8 to 25 guts were transferred to small glass ampoules which were sealed under nitrogen and stored at −20°C until analysis with a Finnigan 4021 gas chromatograph–mass spectrometer. GC capillary columns used were 25 m × 0.2 mm ID or 50 m × 0.35 mm ID of fused silica coated with OV-351 (Alltec Assoc.), film thickness 0.57 μm, or with Superox FA (Supelco Inc.), film thickness 0.37 μm, respectively. The terpenoid compounds analyzed in this study elute in the same order from both stationary phases. Helium was used as carrier gas at a velocity of 0.3 m/sec. The injector temperature was 210°C. After injection, the GC oven temperature remained at 50°C for 4 min and then was increased to 230°C at 8°C/min.

Heptyl acetate (30 ng/μl pentane) was added as an internal standard to the extraction solvent for quantitative calculations and comparisons of retention characteristics. Heptyl acetate was chosen as its retention time neither interferes with monoterpene hydrocarbons nor their oxygenated derivatives on the stationary phases used in this study. In our material, however, the heptyl acetate was to some extent (less than 15%) transformed to 1-heptanol and very small amounts of heptanal. For the quantitative determinations the sum of heptyl acetate, heptanol and heptanal were assumed to equal the amount of heptyl acetate originally added. A response factor of 1 was used for the quantification of the

TABLE 1. DESCRIPTION OF ATTACK PHASES, MATING, AND FEMALE EGG NUMBERS

Attack phases	No of beetles collected[a]				Females mated (%)		Eggs in ovaries ($\bar{X}$)[b]	
	T. minor		*T. piniperda*					
	♂	♀	♂	♀	*T. minor*	*T. piniperda*	*T. minor*	*T. piniperda*
Hibernating	0	0	49	32	—	28	—	0
Walking on host bark	10	8	14	26	25	46	0	0
Single beetles in tunnel, boring just started	0	69	22	26	49	81	0.1	0
Pairs of beetles in tunnels[c]	93	106	—	—	79	—	0.2	—
1. male waiting at entrance	—	—	79	55	—	87	—	0
2. male inside tunnel	—	—	40	47	—	81	—	0
3. as 2. and resin contacted	—	—	33	34	—	85	—	0
Nuptial chamber formed	42	46	166	138	93	97	1	0.7
Egg laying								
Galleries <2 cm	17	17	0	0	100	—	1.3	—
Galleries >2 cm	23	21	55	56	100	100	1.3	0.8
	185	267	458	414				
	452		872					

[a] All insects collected were used both for studies of mating and egg content and for analyses of volatiles in guts.
[b] 0–3 eggs in *T. minor*, 0–2 eggs in *T. piniperda*.
[c] Attack phase "Pairs" in *T. piniperda* was separated into three subphases according to the length of the tunnel and the position of the male, as this species was sampled from thicker bark than *T. minor*. In the smaller species, formation of pairs proceeds more rapidly due to the thinner bark.

oxygenated monoterpenes relative to the internal standard. At least 0.05 ng/extract was required for a reliable quantification of identified compounds.

Chemicals. For all identified compounds, synthetic references were available so that relative retention times and mass spectra could be compared with the compounds in the insect extracts. Myrtenal was obtained from myrtenol by treatment with chromium trioxide dipyridine complex in acetic acid (Baeckström, 1978) and analyzed immediately after synthesis. (1*S*,6*R*)-3-Carene-10-ol was obtained for reference by lithium aluminium hydride reduction of chaminic acid from the cypress *Chamaecyparis nootkatensis* (Lamb.).

The chemicals used for electroantennography, laboratory bioassay, and field trapping were 2-methyl-3-buten-2-ol, 97%; (*R*/*S*)-ipsenol, 85%; (+)-(1*R*,4*S*,5*R*)-*trans*-verbenol here called (*R*)-*trans*-verbenol, containing 12% *cis*-verbenol and <0.1% verbenone; (−)-(1*S*,4*R*,5*S*)-*trans*-verbenol here called (*S*)-*trans*-verbenol, containing <5% (*R*)-isomer and 12% *cis*-verbenol and 1% verbenone; (*R*/*S*)-*cis*-verbenol, containing 12% *trans*-verbenol; (*R*/*S*)-verbenone, 80%; (−)-*cis*-myrtanol, 95%; 2-phenylethanol, 99%; (*R*/*S*)-frontalin, >95%; (*R*/*S*)-*exo*-brevicomin, >95%; (−)-(1*S*,5*S*)-α-pinene and (+)-(1*R*,5*R*)-α-pinene here referred to as (*S*)-α-pinene and (*R*)-α-pinene, 99%; (1*S*,6*R*)-3-carene, $[\alpha]_D^{20} = +21°$; terpinolene, >99%; and myrcene, >99.8%. The chemical standards were obtained from Aldrich Chem. Comp., Steinheim, FRG, Borregaard Ind. Ltd, Sarpsborg, Norway, Chem. Samples Co., Ohio, US, or Fluka AG, Buchs, Switzerland.

Synthesis of (1S,6R)-3-Carene-10-ol for Biological Tests. Gollnick et al. (1965) and Gollnick and Schade (1966) have described a synthesis of (1*S*,6*R*)-3-carene-10-ol (7) from (1*S*,6*R*)-3-carene and their scheme was followed, although each step in the sequence was modified (see Figure 1). The photooxidation of 3-carene (1) was carried out in the presence of $TBABH_4$ according to Baeckström et al. (1982), and the crude reaction mixture was subjected to medium-pressure liquid chromatography (MPLC) on silica gel. The fractions containing monooxygenated products were combined and selectively acetylated by treatment with equal amounts of acetic anhydride and pyridine at room temperature. The acetate (5) of alcohol (4) was isolated by MPLC on silica gel and hydrolyzed with KOH in methanol. This gave the crystalline alcohol (4) which was transformed to the aldehyde (6) by an oxidative rearrangement using PCC and pTSOH in dichloromethane (Baeckström et al., 1982). Reduction of the aldehyde (6) with LAH gave the desired alcohol (7). The absolute configuration of the enantiomers of acid (8) have been determined. The (+)-(1*S*,6*R*)-enantiomer is called chaminic acid and the (−)-(1*R*,6*S*)-enantiomer is called isochamic acid (Norin, 1964). The aldehyde (6) was oxidized to the corresponding acid with sodium chlorite (Lindgren and Nilsson, 1973), and its specific rotation, [^{1}H]NMR spectrum, and melting point were found to be the same as those of chaminic acid (8) [m_p = 104.5–105.5°C, Lit. = 103–106°C, $[\alpha]_{589} = +7.2°$ (c = 3.4, MeOH), Lit. = +6 (c = 3.9, MeOH)].

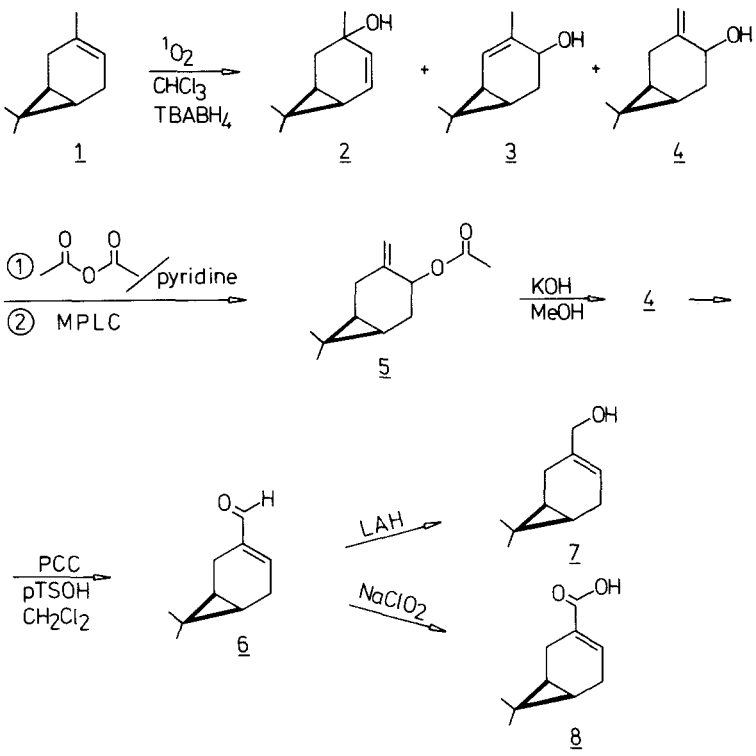


FIG. 1. Reaction scheme for synthesis of 3-carene-10-ol (7) from 3-carene (1).

Electroantennography (EAG). Antennae were prepared according to Van Der Pers et al. (1984). The EAG responses were monitored on an oscilloscope, and maximum amplitudes recorded by a digital voltmeter as described by Bjostad and Roelofs (1980). The stimulation technique was essentially the same as that described by Van Der Pers (1981). A piece of filter paper (2 cm^2), with the test compound was put into a disposable 5-ml plastic syringe. No correction was made for differences in volatility among the test compounds. For each test run on an antenna the "mean response" was defined by subtracting the blank response from all measurements and then calculating the mean of the resulting amplitudes from 12 straight-chain alcohols (methanol to dodecanol), 2-methyl-3-buten-2-ol, ipsenol, *cis-* and *trans*-verbenol, verbenone, myrtenol, *cis*-myrtanol, phenylethanol, frontalin, and *exo*-brevicomin. All EAG responses are expressed as percent of this "mean response."

Laboratory Bioassay of Walking Beetles. T. minor were excise from windthrown pine trees two to three days after the first dispersal period. *T. piniperda* were picked during dispersal days while they were walking on pine trunks in a forest 30 km east of Lund, in southernmost Sweden. Beetles were

kept in the dark, 100% relative humidity, 4°C until used for testing. The open arena bioassay was as described by Byers and Wood (1981). Ten beetles at a time were released 20 ± 2 cm from the odor source on an open arena with a laminar air flow, approximately 0.6 m/sec. A beetle was recorded as attracted if it had approached closer than 1 cm from the odor source within 2 min after release. Unresponsive beetles were given a second try. Odors from pine logs for bioassay were obtained either directly by pumping air through a glass jar with a log, or indirectly after adsorption on Porapak Q and diethyl ether solvent extraction. In the latter case the adsorbed components were fractionated with capillary GC as described by Byers et al. (1985). The fractionated host compounds and the synthetic compounds were released from 5-μl glass capillaries placed in the air stream.

Field Trapping. The attractivities of a mixture of the racemic *trans*-verbenol and (1*S*,6*R*)-3-carene-10-ol as well as host odors were tested in three field-trapping experiments. The monoterpene alcohols were used in three different baits: alone (MT-OH), together with fresh pine logs (Log + MT-OH), and together with purified host monoterpenes (MT-OH + MT). The host monoterpenes used, α-pinene, terpinolene, and 3-carene, had earlier been found to be attractive to *T. piniperda* (Byers et al., 1985).

Trapping was done in a homogeneous, evenly aged (about 70 years) pine stand 30 km east of Lund, Sweden, in April–May in 1983 and 1984. The trap logs were covered with brass nets (No. 60) to prevent arriving beetles from boring. Logs and vials with synthetic terpenes were surrounded by sticky trap, hardware cloth (No. 4), and coated with Stickem Special® (Byers and Wood, 1980). The monoterpene alcohols were each released from a 1-ml polyethylene vial (Kartell, Italy), loaded with 50 μl of the compound. The release rate of (*R*)-*trans*-verbenol from such vials was estimated by weight loss over 21 days in the laboratory to be 0.20 mg/day ($N = 8$, $r = 0.999$, hole size 4.2 mm, 20°C). The release rates of α-pinene, 3-carene, and terpinolene were each about 30 mg/day at 18°C. Treatments were assigned at random to traps placed on steel poles 1.5 m above ground and 10 m apart in a line. Rearrangement of treatment traps and collection of beetles were performed after at least 15 beetles were caught on the most attractive trap. The catches from 1984, 12 replicates, were transformed according to log (catch + 0.25) which gave homogeneous variances before ANOVA followed by Duncan's multiple-range test.

RESULTS

Attack Phases, Mating, and Egg-Laying. When walking on the host tree before entering the bark, 25% of *T. minor* and 46% of *T. piniperda* females were mated, and in both species at least 90% were mated when the nuptial chambers had been constructed (Table 1). In *T. piniperda* no fully developed

eggs could be found in the ovaries until after the nuptial chambers had been completed, while in *T. minor* some beetles contained eggs just after they had started boring ($\bar{X}$ = 0.1 eggs per female). When construction of egg galleries had begun, the mean number of eggs per female was 1.3 for *T. minor* and 0.8 for *T. piniperda*.

Volatile Compounds in Hindgut. The major volatiles found in the hindguts of the two pine shoot beetles were oxygenated monoterpenes (Table 2). In addition, smaller amounts of monoterpene hydrocarbons and sesquiterpenes were found. In *T. minor*, both the number of compounds and the amounts of detectable oxygenated monoterpenes were lower than in *T. piniperda*. Three compounds, 3-carene-10-ol, *trans*-verbenol, and myrtenol, dominated in both sexes of the two species (Table 2). Less than 2 ng per beetles of *cis*-verbenol, verbenone, and myrtenol were present in *T. minor* and less than 4 ng per beetle of verbenone, 3-carene-10-al, myrtenal, and borneol were found in *T. piniperda*. Small amounts (<5 ng/beetle) of a few unidentified oxygen-containing monoterpenes were sometimes found, and none of these compounds were detected in both species.

In general the amounts of the oxygenated monoterpenes reached a maximum in both species when the beetles had bored through the outer bark and contacted the resin-containing phloem and xylem. In the phases following construction of the nuptial chamber, the amount of oxygenated terpenes decreased and continued to decline during egg-laying. This pattern is illustrated in Figure 2A–C for the three major oxygenated terpenes, *trans*-verbenol, 3-carene-10-ol, myrtenol, and for verbenone (Figure 2D).

TABLE 2. OXYGENATED MONOTERPENES IN GUT OF PINE SHOOT BETTLES WHEN BORING IN RESIN-CARRYING TISSUE (ATTACK PHASE: "PAIRS")

	Monoterpene, ng/beetle (mean value ± standard deviation)			
	Tomicus minor		*Tomicus piniperda*	
Compound	93 ♂ $N = 5^a$	100 ♀ $N = 6^a$	73 ♂ $N = 4^a$	81 ♀ $N = 5^a$
trans-Verbenol	2.8 ± 2.0	6.8 ± 5.8	6.8 ± 0.9	6.4 ± 1.2
cis-Verbenol	0	0.3 ± 0.3	0	0
Verbenone	1.0 ± 0.4	0.4 ± 0.3	3.5 ± 0.6	2.3 ± 0.5
3-Carene-10-ol	11.6 ± 5.9	7.2 ± 5.9	27.9 ± 10	10.9 ± 3.5
3-Carene-10-al	0	0	0.3 ± 0.3	0.4 ± 0.1
Myrtenol	1.6 ± 1.0	1.4 ± 1.4	15.6 ± 4.4	9.4 ± 2.2
Myrtenal	0	0	0.6 ± 0.2	0.5 ± 0.2
Borneol	0	0	1.6 ± 0.8	1.0 ± 0.3

[a] N = number of extracts into which the beetles were divided at analysis.

The guts of *T. minor* females on the average contained more *trans*-verbenol than their conspecific males (Figure 2A). *T. piniperda* males, compared to the females, generally seemed to contain somewhat larger amounts of all the terpenes. However, it should be noted that the range of variation of samples prepared from the same sex and attack phase exceeds the differences between the sexes.

To determine the influence of various host trees on the content of oxygenated monoterpenes in *T. piniperda*, beetles were separated and extracted according to the specific tree they attacked. Table 3 shows the chemical analyses of beetles from the phase in which nuptial chambers have been formed. The insects collected from trees A and D contained significantly larger amounts of oxygenated monoterpenes than those collected from tree B and log C ($P < 5\%$, Wilcoxon signed-ranks).

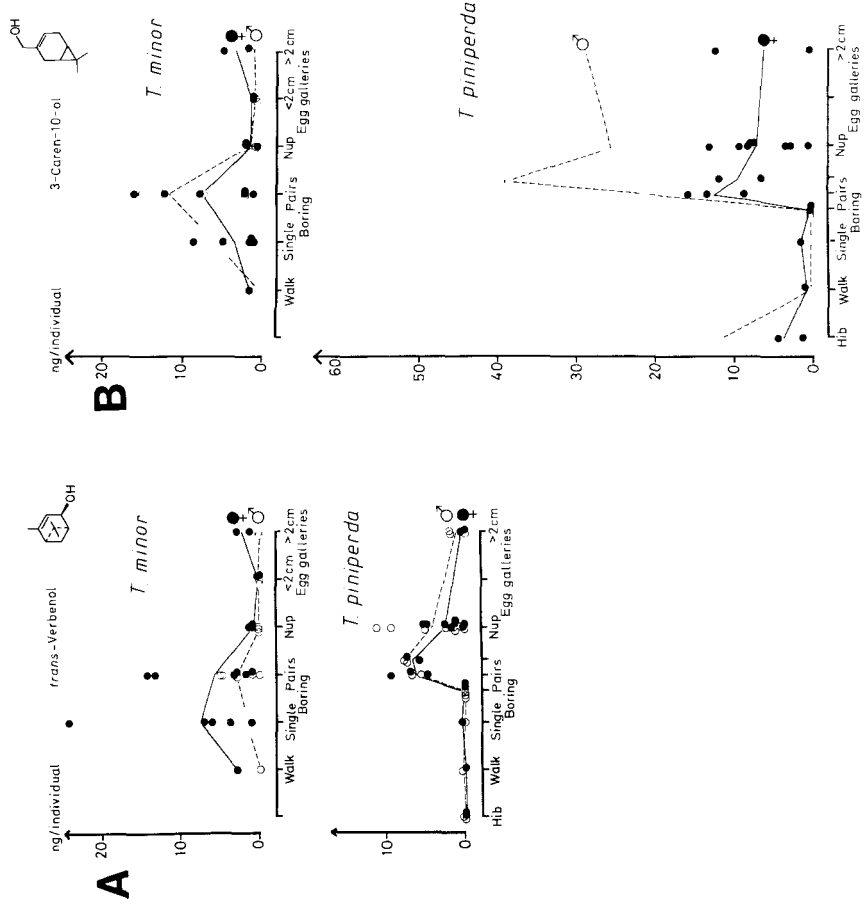
Electroantennography. As a first step in determining the biological activities of the compounds identified in beetles, we recorded EAG responses in both species (Figure 3). In addition, we tested the response to some compounds attractive to other Scolytidae, as well as some host pine monoterpenes (Figure 3), and a homologous series of 12 primary straight-chain alcohols (methanol to dodecanol, not shown in the figure) was included. The straight-chain alcohols evoked a similar response pattern in both species and sexes. The responses increased with chain length to a maximum between pentanol to heptanol and then decreased with further increase in chain length. The amplitude of these responses to the alcohols were of the same order of magnitude as the other compounds tested.

For *T. minor* the strongest response was elicited by *trans*-verbenol followed by *cis*-verbenol. In *T. piniperda*, on the other hand, verbenone evoked the strongest response followed by *cis*-verbenol for the females and *trans*-verbenol for the males. However, the differences in response to *trans*-verbenol and verbenone were not significant either between the species or the sexes ($P < 5\%$, Student's t test).

Of the three major monoterpene alcohols in the beetles guts, *trans*-verbenol evoked a significantly higher response in both species and sexes compared to 3-carene-10-ol ($P < 5\%$, Wilcoxon signed rank), while myrtenol gave an intermediate response. No significant difference was observed for either species when comparing the two enantiomers of *trans*-verbenol at the same dose.

When *T. minor* antennae were exposed to different doses of (1*S*,6*R*)-3-carene-10-ol and the two enantiomers of *trans*-verbenol, both *trans*-verbenols caused signals at least twice as strong as those from 3-carene-10-ol, and they also had lower thresholds (Figure 4). The (*S*)-*trans*-verbenol was slightly more active in the tests than the (*R*)-enantiomer, but the difference was not significant.

The antennae of both species were also exposed to some major host tree


A
B
ng/individual
trans-Verbenol
3-Caren-10-ol
T. minor
T. piniperda
Walk
Single
Pairs
Boring
Nup
<2cm
>2cm
Egg galleries
Hib

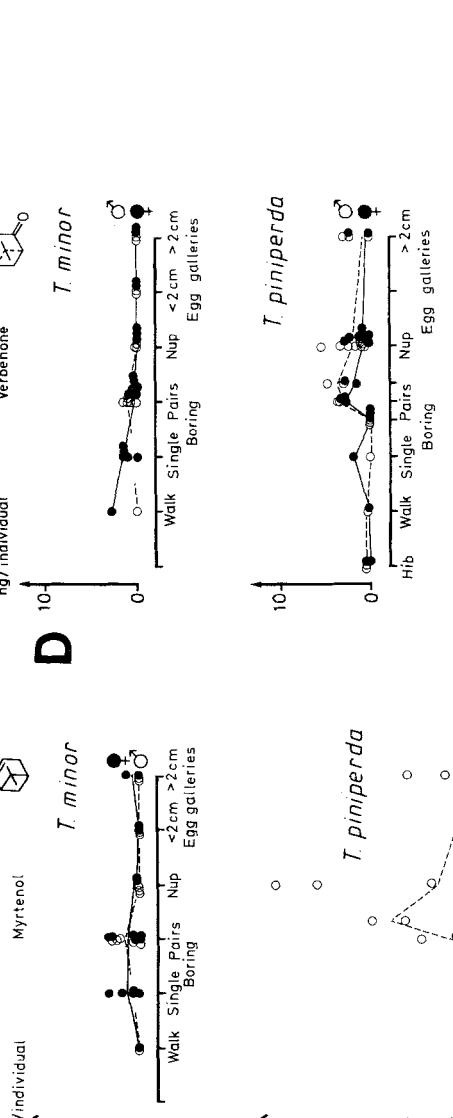


FIG. 2. Amount of (A) *trans*-verbenol, (B) 3-carene-10-ol, (C) myrtenol, and (D) verbenone in guts of *T. minor* and *T. piniperda* in different attack phases. Each point gives the result of an analysis of 8–25 beetles, expressed as nanograms compound per beetles. The broken lines for males and solid lines for females connect the mean amounts for all beetles of each phase and sex. See Table 1 for description of attack phases.

TABLE 3. OXYGENATED MONOTERPENES IN GUTS OF *T. piniperda* ATTACKING FOUR DIFFERENT TREES (ATTACK PHASE: NUPTIAL CHAMBER FORMED)

Compounds		Monoterpenes (ng compound/beetle) Tree A ♂ $N = 50$ ♀ $N = 47$	Tree B ♂ $N = 43$ ♀ $N = 39$	Tree C ♂ $N = 55$ ♀ $N = 33$	Tree D ♂ $N = 15$ ♀ $N = 19$
trans-Verbenol	♂	7.59	2.81	0.80	9.33
	♀	3.06	1.80	0.47	4.95
Verbenone	♂	3.77	1.57	0.68	3.29
	♀	1.24	0.66	0.14	2.12
3-Carene-10-ol	♂	45.8	22.8	7.18	32.8
	♀	6.50	10.3	2.50	7.78
3-Carene-10-al	♂	0.48	0.36	0.13	1.41
	♀	0.25	0.21	0.00	0.27
Myrtenol	♂	22.8	11.1	4.94	27.4
	♀	5.80	6.49	3.33	9.9
Myrtenal	♂	0.37	0.28	0.20	0.43
	♀	0.33	0.18	0.00	0.35
Borneol	♂	1.24	0.76	0.22	1.03
	♀	0.58	0.84	0.21	0.58
Unidentified	♂	1.19	0.61	0.16	1.46
	♀	0.38	0.59	0.00	0.48
Unidentified	♂	0.82	0.55	0.44	1.53
	♀	0.37	0.31	0.17	0.72
Unidentified	♂	2.90	2.15	0.78	3.64
	♀	0.74	1.34	0.30	0.98
Σ (ng/beetle)	♂	87	43	15.5	82
	♀	19	23	7.1	28

monoterpenes: (*R*)- and (*S*)-α-pinene, (1*S*,6*R*)-3-carene, terpinolene, and myrcene. The responses to these compounds were generally lower than to the oxygenated monoterpenes. No differences between sexes were found, while *T. piniperda* seemed to respond more strongly than *T. minor* to these host compounds.

Laboratory Bioassay with Walking Beetles. Behavioral tests were carried out under controlled conditions with beetle- and host-produced compounds. In the open-arena olfactometer both sexes of *T. minor* were attracted to odors from a pine log, while introduction of females into the log further increased the attraction of both sexes (Table 4, experiment 1). In contrast, *T. piniperda* beetles

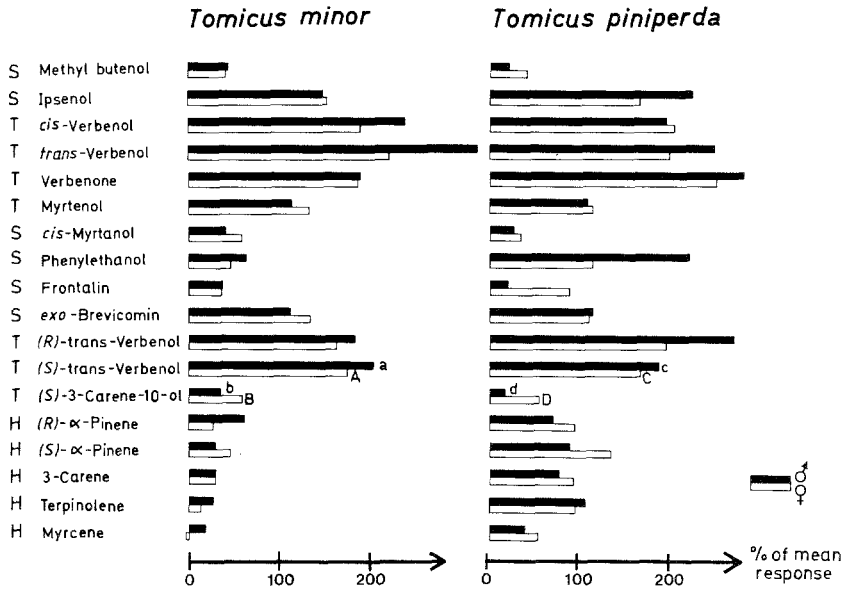


FIG. 3. EAG response of *T. minor* and *T. piniperda*, to a series of oxygenated monoterpenes produced by *Tomicus* (T), other scolytid pheromone substances (S), and host monoterpenes (H). Each insect antenna was exposed to the chemicals in the order they appear in the figure. Each compound (500 μg) was added on filter paper. For the comparison between (*S*)-*trans*-verbenol and (*S*)-3-carene-10-ol, within each species and sex, bars marked with different letters denote significantly different responses ($P < 5\%$, Wilcoxon signed-rank). Sample size was 20/16 (males/females) in *T. minor* (host compound 6/6) and for *T. piniperda* 8/15.

were strongly attracted to the pine log alone with no apparent increase in attractivity when conspecific females were present in the log. In experiment 2, Table 4, the attractiveness of the synthetic compounds, (*R*)- and (*S*)-*trans*-verbenol and (1*S*,6*R*)-3-carene-10-ol, to *T. minor* males was compared and 3-carene-10-ol was not found to be attractive. In experiment 3 the same compounds were tested together with the monoterpene hydrocarbon fraction from pine. Again, the response to (1*S*,6*R*)-3-carene-10-ol was not significantly different from the solvent alone, but the combination of host terpenes and (*S*)-*trans*-verbenol was active. The strongest response (97%) of *T. minor* males was to a combination of a pine log and a low release of synthetic (*S*)-*trans*-verbenol (experiment 4).

Field Trapping. In the first test (Table 5, A1), performed towards the end of the flight period in 1983, indications were found that *T. minor* was attracted by the three alcohols (*R*)- and (*S*)-*trans*-verbenol and (1*S*,6*R*)-3-carene-10-ol alone (MT-OH) and in combination with pine logs. Similarly, a subtractive

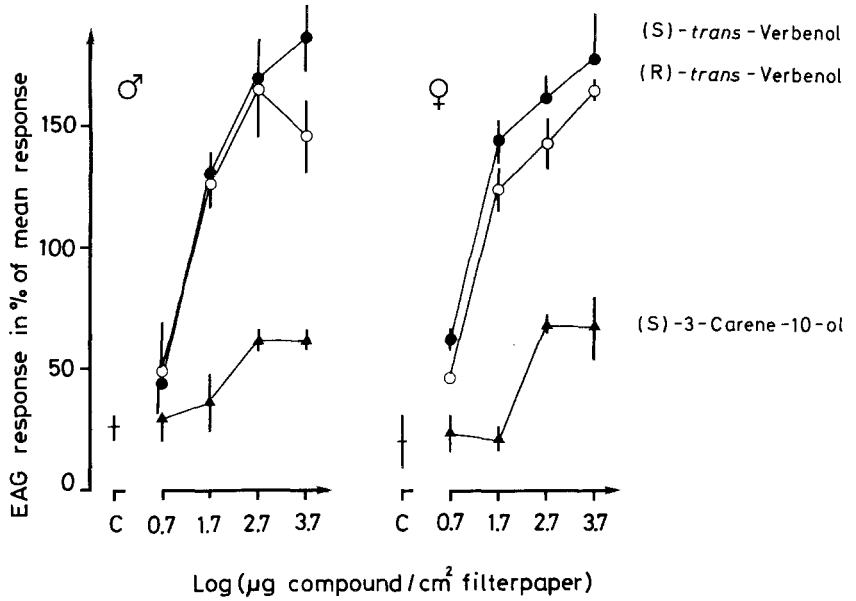


FIG. 4. Mean EAG response of *T. minor* to different doses of (*S*)- and (*R*)-*trans*-verbenol and (1*S*,6*R*)-3-carene-10-ol applied as hexane solutions on a filter paper. Control was hexane alone. Bars represent the standard deviation.

assay of the three alcohols at the end of the 1983 flight period gave no significant differences in attraction of either species between logs baited with (1): MT-OH, (2): (*R*)+(*S*)-*trans*-verbenol, (3): (*S*)-*trans*-verbenol + (1*S*,6*R*)-3-carene-10-ol, or (4): (*R*)-*trans*-verbenol + 3-carene-10-ol. The pooled catch of *T. minor* had 68.1% males ($N = 91$, 95% CI-58–77), while *T. piniperda* catch was 51.2% males ($N = 374$).

The second test (A2) in 1984, started in the middle of the flight period, showed that traps containing *trans*-verbenols, 3-carene-10-ol, and a fresh pine log caught more *T. minor* than any of the other four treatments and was significantly different from a log alone or the blank (Figure 5). The difference in total catch between a log alone (31 beetles) and a log with the monoterpene alcohols (275 beetles) was especially prominent. For *T. minor* the baits containing the monoterpene alcohols alone differed significantly from the blank, while the log alone was not significantly different from the blank.

For *T. piniperda*, all three baits containing either pine log or the host monoterpene hydrocarbons caught significantly more beetles than the blank. The bait with the monoterpene alcohols alone did not catch more than the blank. An increase in catch was obtained when the monoterpene alcohols were added to the log, but this increase was not statistically significant.

TABLE 4. ATTRACTIVITY OF NATURAL AND SYNTHETIC VOLATILES IN LABORATORY BIOASSAY

Stimulus	Beetles responding, % (N = 30)			
	Tomicus minor		*Tomicus piniperda*	
	Male	Female	Male	Female
Experiment 1				
Pine log	20 " Ψ[c]	17 " Ψ	73 " Ψ	67 " Ψ
Pine log infested with 30 conspecific females	90 § Ψ	87 §	63 " Ψ	63 "
Experiment 2				
(*S*)-*trans*-verbenol + (*R*)-*trans*-verbenol[a]	47 §			
(*S*)-3-carene-10-ol	7 "			
(*S*)-*trans*-verbenol	27 " §			
(*R*)-*trans*-verbenol	23 " §			
Experiment 3				
Pine fraction[b]	20 "			
Pine fraction + (*S*)-*trans*-verbenol	57 §			
Pine fraction + (*R*)-*trans*-verbenol	33 " §			
Pine fraction + (*S*)-3-carene-10-ol	7 "			
Experiment 4				
Pine log	67 "	40[d] "		
Pine log + (*S*)-*trans*-verbenol	97 §	60 "		

[a] 220 ng/min of monoterpene alcohols in diethylether were released from capillaries in exp. 2 and 3; and 70 ng/min released from a closed 1-ml PE vial in exp. 4.

[b] Monoterpene hydrocarbon fraction in diethyl ether after entrainment and GC fractionation.

[c] ", §, Values followed by the same symbol are not significantly different within a species and sex by chi-square corrected for continuity at $P < 5\%$ (significance level adjusted for number of comparisons) in each experiment. Ψ, Response different between species within sex by chi-square corrected for continuity at $P < 5\%$.

[d] 40 females tested.

T. minor males were caught in a significantly higher proportion than females in the traps containing both a log and the monoterpene alcohols in all three tests (Table 4). For *T. piniperda*, on the other hand, no sex ratios significantly differed from unity.

DISCUSSION

Pheromone Activity in Tomicus. In *T. piniperda* (including *T. destruens* Woll.), Schönherr (1972) and Carlé (1974, 1978) report indications of pheromonal attraction to beetle infested logs. However, appropriate statistical tests and controls are lacking in these three studies. Kangas et al. (1970a) and Carlé

TABLE 5. SEX RATIO OF *T. minor* AND *T. piniperda* CAUGHT AT STICKY TRAPS SURROUNDING LOGS OR SYNTHETIC BAITS, SPRING 1983 AND 1984, SKÅNE, SWEDEN.

	Sex-Ratio in Catch					
	T. minor			*T. piniperda*		
Baits[a]	Sample size[b]	Males (%)	95% CI[c]	Sample size[b]	Males (%)	95% CI[c]
Test A1, activity of pheromone, April 29–May, 19, 1983, 6 replicates						
Log +MT-OH	96	62.5[d]	53–72	124	48.7	38–56
MT-OH	50	76.0[d]	63–86	26	42.3	26–61
Log	12	(50)[e]	25–75	76	47.4	37–58
Blank	4	(50)[e]	15–85	22	59.1	39–77
Test A2, activity of pheromone, April 14–27, 1984, 12 replicates						
Log +MT-OH	275	60.1[d]	54–66	473	50.6	46–55
MT-OH	86	48.3	38–59	32	61.3	44–76
MT +MT-OH	75	58.7	47–69	166	56.8	50–65
Log	31	56.2	39–72	294	54.0	49–60
Blank	4	(50)[e]	15–85	21	60.0	37–76

[a] Bait designations: MT-OH = (*S*)-*trans*-verbenol + (*R*)-*trans*-verbenol + 3-caren-10-ol, oxygenated monoterpenes released from separate vials at approx. 0.2 mg/day, see Methods. MT = (*S*)-α-pinene + (*R*)-α-pinene + 3-carene + terpinolene, monoterpene hydrocarbons released from separate vials at approx. 30 mg/day (Byers et al., 1985), see Methods.

[b] Sample size equal to or less than total catch, as maximum 100 individuals were sexed from each replicate and some individuals were too damaged to sex.

[c] Binomial 95% confidence interval (Byers & Wood 1980).

[d] Value significantly different from an equal sex ratio.

[e] Value calculated on less than 20 individuals.

(1978) in laboratory bioassays claimed *trans*-verbenol to be attractive, but no statistics were presented. On the other hand, field-trapping experiments by Perttunen et al. (1970) did not find that the presence of boring females (assumed to be the pheromone-producing sex) enhanced the attractiveness of logs. The results of the present study complement these results and those of Byers et al. (1985), who developed a systematic method for isolating semiochemicals from pine logs infested with *T. piniperda* (sexes together or each alone) and found no evidence for a long-range pheromone for mate and host location. Instead, pine volatiles, (*R*)- and (*S*)-α-pinene, 3-carene, and terpinolene, were found in field and laboratory experiments to be attractants (Byers et al., 1985). These three compounds were later shown to induce attacks by *T. piniperda* on vigorous trees, unsuitable for brood development (Schroeder and Eidmann, 1987). Kangas et al. (1965) and Oksanen et al. (1968) concluded from laboratory bioassays (without any statistical analysis of data) that *cis*- and *trans*-carveol

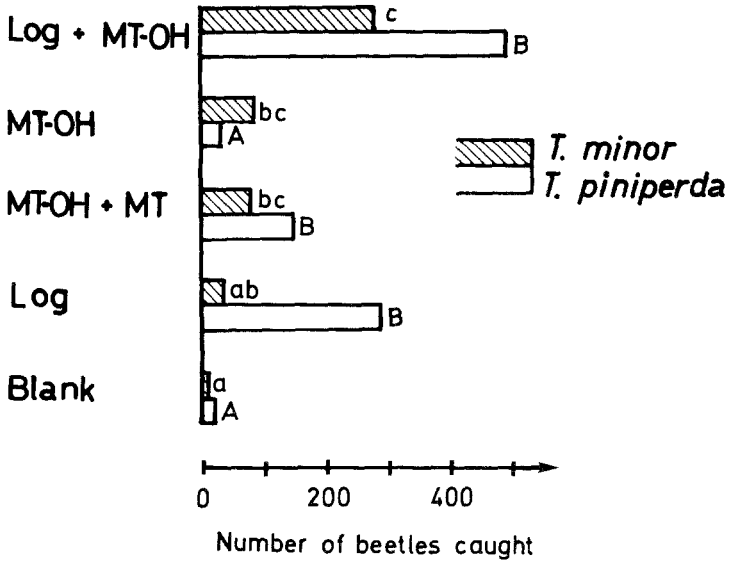


FIG. 5. Catch of *T. minor* and *T. piniperda* on sticky traps with five different baits. The lengths of the bars correspond to the total catch in 12 replicates in 1984. Bars followed by the same lower or upper case letters form homogeneous subsets [$P < 5\%$, Duncan's multiple-range test after ANOVA on log (catch + 1/4)]. Baits marked with MT-OH contain (*S*)- and (*R*)-*trans*-verbenol and (1*S*,6*R*)-3-carene-10-ol and those marked with MT contain (*S*)- and (*R*)-α-pinene, terpinolene, and (*S*)-3-carene.

together with α-terpineol were the most active host compounds and thus responsible for "primary attraction." However, their bioassays were performed in closed-choice chambers with no odor gradient at equilibrium. In our open-arena walking bioassay the odor is mixed with a stream of air. This allows both an odor gradient and a wind direction in which the beetles can orient and thus simulates the natural condition more closely.

The existence of a pheromone in *T. minor* produced by the female is indicated by the female initiated galleries, the strong bioassay response of both sexes to female infested logs, and the >50% males caught at traps baited with (*S*)- and (*R*)-*trans*-verbenol and 3-carene-10-ol. It is, however, possible that males also may contribute to the pheromonal attraction as their production of the three alcohols is almost as large as that of the females (Table 2). In a large sample of pine shoot beetles attracted to pine logs or feeding in pine shoots, Långström (1980, 1983) found an equal or slightly female biased sex ratio for both species in middle Sweden. Thus the higher percentage of males of *T. minor* at pheromone traps probably does not reflect the mean sex ratio of the population. In contrast, *T. piniperda* did not respond more to female infested logs than

to uninfested logs, and the sex ratio at traps baited with the alcohols was not different from unity.

The fact that a long-range pheromone has not been found for *T. piniperda* in this or other studies is notable, as pheromone communication in scolytid biology has almost become a paradigm (Borden, 1982; Wood, 1982). However, in at least two other cases research has failed to prove a pheromonal attraction but instead an attraction to host odors (*Pseudohylesinus nebulosus*; Ryker and Oester, 1982) or to odors of a decaying host (*Hylurgopinus rufipes*; Gardiner, 1979; Lanier, 1983). *P. nebulosus* is similar to *T. piniperda* in several respects: not only does it disperse early in spring and attacks only felled or weakened trees, but logs with male and female *P. nebulosus* were also somewhat more attractive than logs alone, parallel to the findings of Byers et al. (1985). In each of these studies the increase in attraction was attributed to a large release of host volatiles from logs with excavating beetles rather than to a weak pheromonal attraction.

Semiochemical Production and EAG Responses. Both the (*R*)- and (*S*)-isomers of *trans*-verbenol were tested in the biological experiments because the absolute configuration of the beetle produced compound was not determined. In the EAG tests with *T. minor*, *trans*-verbenol induced the largest total response and the (*S*)-enantiomer the highest dose–response. Bakke and Jordal (personal communication) also found indications that (−)-(*S*)-*trans*-verbenol acts as an attractant for *T. minor* in field-trapping experiments. When considering the activity of *trans*-verbenol, the relatively large impurity of *cis*-verbenol could be important. In fact, the amount of *cis*-isomer (about 10%) in the synthetic *trans*-verbenol used in our field tests is about as large as the ratio of *cis* to *trans* naturally found in hindguts of *T. minor*. Thus, the studies reported here do not uniequivocally support *trans*-verbenol as the only active component in the *T. minor* pheromone.

Only the (1*S*,6*R*)-enantiomer of 3-carene-10-ol was used in laboratory and field experiments since its precursor, 3-carene of the wood oil in Scots pine, is known to possess the (1*S*,6*R*)-configuration (Semmler and von Schiller, 1927; Aschan, 1928; Norin, 1964). 3-Carene-10-ol has earlier been identified in the mountain pine beetle, *Dendroctonus ponderosae* (Conn et al., 1983), but the absolute configuration was not determined and the compound was not found to have any effect on field trap catches. Our results with the laboratory olfactometer and EAG also indicate that 3-carene-10-ol is not an essential attractive pheromone component in *T. minor*.

The demonstration of the presence of *trans*-verbenol, myrtenol, and verbenone in *T. piniperda* hindguts is in agreement with earlier studies (Francke and Heeman, 1976; Carlé et al., 1978). However, Francke and Heeman's observation that virgin females and males have relatively high amounts of verbenone was not confirmed in our study. The amounts of verbenone were highest

in both sexes just before the construction of egg niches and at that time most females were mated.

The value of electrophysiology as a screening method for attractive pheromone candidates in bark beetles is probably limited. For example, it is known that bark beetles can have receptors highly sensitive to pheromone components of other species which act as allomones (Light and Birch, 1982). In fact, we found significant EAG activity in both *Tomicus* species to several bark beetle pheromone compounds that were not detected in either *Tomicus* species. Our study also showed that several monoterpenes attractive to *T. piniperda* gave smaller EAG responses than *trans*-verbenol and other oxygenated monoterpenes. These oxygenated compounds could, however, elicit other, as yet undiscovered, pheromonal activities at close range.

In the hindgut extracts, large amounts of 1-heptanol were found. When 1-heptanol, a pheromone component of *Dendroctonus vitei* (Renwick et al., 1975), and some homologs were at first included in the EAG tests, both species were almost as sensitive to these straight chain alcohols as to the oxygenated monoterpenes. However, it was later found, by addition of hexyl and octyl acetate to the gut extraction solvent, that the heptanol originated from transformations of the heptyl acetate that was used as an internal standard. Short-chain alcohols are also known to evoke EAG response in the Colorado beetle (Visser, 1979) and some lepidopteran species (Van Der Pers, 1981).

Evolutionary Considerations. How can the two *Tomicus* species be placed in a larger evolutionary framework of olfactory orientation during host colonization in bark beetles? The first "primitive" system to evolve may have relied only on host plant compounds for host location and acceptance, as in many phytophagous insects. As the use of long-range aggregation pheromones evolved, the need to utilize host attractants could have decreased in significance. In most insects, the egg-laying potential of the female is the most critical resource for reproduction, and females produce a sex pheromone attracting only males from a distance. In some insect groups, males produce pheromone and provide essential resources for reproduction like nuptial gifts in scorpionflies (Bornemissza, 1964) or announce clumped resources for reproduction, as in some stored-product beetles (Burkholder and Ma, 1985) and in polygynous bark beetles (Kirkendall, 1983). Female-produced aggregation pheromones, however, seem to be restricted to bark beetles and function in host-killing cooperation to establish a food resource after successful colonization of a tree.

Possibly, an early step in pheromone system evolution was represented by groups that modified host compounds for use as pheromone components (many *Ips*; Wood, 1982). More advanced systems have complex bicyclic ketals (*Dendroctonus*, *Pityogenes*) or tricyclic ketals (*Trypodendron*, *Scolytus*) of which the carbon skeletons are not found in the host. These compounds are, however, usually still synergized by host compounds (Borden, 1982; Wood, 1982). Other

advanced systems include loss of host synergism but with monoterpene alcohols in conjunction with short-chain alcohols (*I. typographus*; Bakke et al., 1977; *I. cembrae*; Stoakley et al., 1978). The small difference between the sexes of *T. minor* in the production of the major component *trans*-verbenol indicates a primitive system not far evolved from a simple detoxification of host terpenes.

Both *Tomicus* species apparently have a similar production and sensory perception of oxygenated compounds suitable as chemical messengers but *T. piniperda* has not acquired (or maintained) a pheromone system. The life history of the two species differs radically in the mode of feeding by the larvae. In the genus *Tomicus*, three or four palearctic species exist (Schedl, 1946), but of these *T. minor* is the only species which has evolved the fungus-feeding habit (xylomycetophagy; Kirkendall, 1983). This fungus feeding may have allowed an expansion or shift of the spatial niche to the thin-barked sections of the pine, where *T. piniperda* cannot reproduce readily. The xylomycetophagus population may have avoided gene flow from the phloem-feeding population by beginning to use a long-range pheromone. The fact that *T. minor* attacks thin bark (often <0.5 mm) and thus contacts resinous tissue more rapidly would make it easier for this species to develop a "frass pheromone" (Vité et al., 1972), which could be released comparatively quickly (within a day). In contrast, *T. piniperda* may take from one to several days during the early spring conditions (low day-time temperature) to penetrate the thicker cortex bark, and by this time most beetles have landed on the tree in response to host monoterpenes. On the other hand, *T. minor* is reputed to colonize standing, weakly stressed trees more often (Postner, 1974), presumably with the aid of its aggregation pheromone. A further evolution towards a more rapid acting pheromone system might allow *T. minor* to attack viable host trees and expand its niche considerably.

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