Olfactory Recognition of Host-Tree Susceptibility by Pine Shoot Beetles

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Storm-fallen Scots pines with broken roots and trees with severed tops exude wound oleoresin. These trees are susceptible to bark beetles due to an injured vascular system that can not provide adequate oleoresin in order to resist new attacks by beetles [1]. Once a tree is attacked, most bark beetles use pheromone attractants to locate mates and often to overcome tree resistance through a mass attack [1, 2]. It would be clearly advantageous for bark beetles to have evolved sensory systems for efficiently locating their host and in recognizing whether a particular host was less resistant than most healthy trees. However, little is known of how bark beetles select their host from among other plant and tree species [1], or what may attract the first individuals to a susceptible host. We have investigated the semiochemical basis of the mass aggregation of pine shoot beetles, *Tomicus piniperda*, on storm-injured Scots pine. We found that the beetle can recognize while still in flight a host tree and whether it is susceptible by means of olfaction of three plant monoterpenes evaporating from wound oleoresin.

Scots pine logs infested with males and females of *T. piniperda* were tested in the forest of southern Sweden (Table 1, 1982) to determine their attractiveness to naturally swarming populations in the spring. The resulting catches of beetles indicate that there was a strong attraction to volatiles released from logs

alone. Furthermore, the presence of feeding beetles of either or both sexes did not enhance attraction as would be expected if there was a pheromone present.

To identify the attractive host compounds, odors from infested or uninfested logs were collected on Porapak Q (50–80 mesh) and then extracted with diethyl ether and fractionated by capillary gas chromatography (GC) as shown in Fig. 1. The behavioral activities (upwind walking attraction) of various fraction combinations were tested in a laboratory olfactometer [3]. Each extract was fractionated three times (Fig. 1) and for each fractionation behavioral tests were performed in which each of the fractions was subtracted from the test combination in order to observe a loss in activity that would indicate an active component in the subtracted fraction. Using this subtractive bioassay method we found activity only in fraction I (53–67% response) regardless of whether the extract was obtained from infested or uninfested logs. Fraction I contained three monoterpenes, α-pinene, 3-carene, and terpinolene which each alone elicited about 30% response compared to controls of 3 to 10% (each N-60, $P<0.01$, χ^2). α-Pinene from Scots pine contains about an equal ratio of $(+)$ and $(-)$ enantiomers [4]. The identifications were confirmed by comparison to authentic samples by GC-mass spectrometry (Fig. 1).

Commercial samples of $(+)$- and $(-)$-α-pinene ($|\alpha|_D^{20} = +48°$, $-42°$), $(+)$-3-carene ($|\alpha|_D^{20} = +17°$), terpinolene (all >99%, Fluka) and myrcene

(>99.8%, Chemical Samples Co.) were tested in the olfactometer. Both sexes responded similarly to a concentration range of each of the above monoterpenes, although myrcene was not attractive (Table 2). The attraction of *T. piniperda* occurred only at relatively high amounts of monoterpene release (10^{-6} to 10^{-5} g/min, the higher release is comparable to our log) compared to much lower amounts of pheromone components (10^{-9} to 10^{-8} g/min) that are needed to elicit similar responses in other bark beetles [3, 5]. This difference is probably an evolutionary consequence of quantitatively more monoterpene release from damaged trees compared with pheromone release from insects [2, 5].

The host monoterpenes were further tested for attractivity in the forest at release rates comparable to that of each from the Scots pine log. Compared to blank controls, each of the monoterpenes as well as various two- and three-way combinations caught significantly more beetles (Table 1, 1983). The catch on logs was not significantly different from the combination of the three monoterpenes identified above (Table 1, 1983). Our findings are in conflict with earlier reports which suggested that α-pinene and 3-carene are repellents and that α-terpineol and other synergists (in our fractions III–IV) are attractants [6].

T. piniperda is the most important insect pest of pines in Northern Europe and Scandinavia [7]. The beetle typically swarms in the spring before any other bark beetle species that infest pines, and so appears to successfully

Table 1. Attraction of flying *Tomicus piniperda* to traps releasing volatiles from Scots pine logs (28 cm × 13 cm diam.) or from glass tubes containing pine monoterpenes (each >99 percent and released at about 30 mg/day at 18 °C) in Scots pine plantations of southern Sweden

Trap contents[a]	Catch on traps		
	Male	Female	Total[b]
March–April 1982 *(22 replicates each treatment)*			
Blank control	21	31	52[c]
Log	309	314	623[d]
Log+30♀♀	325	315	640
Log+30♂♂	319	313	632
Log+30♀♀+30♂♂	379	395	774
April–May 1983 *(17 replicates each treatment)*			
Blank control	5	2	7[e]
A=(+)-3-Carene	23	25	48
B=(+)-α-Pinene	29	31	60
C=(−)-α-Pinene	38	41	79
D=Terpinolene	57	47	104
A+B	24	29	53
B+C	54	53	107
A+B+D	107	96	203[f]
Log	127	129	256

[a] Methods of infesting and screening of logs and design of sticky traps were conducted as described previously [9]. Treatments in traps (two of each type) were spaced at least 11 m apart and randomized for each replicate period (>1 h each)
[b] None of the sex ratios differ significantly from 1 ($\alpha=0.05$, χ^2)
[c] Catch differs significantly from others ($P<0.001$, $\alpha=0.007$)
[d] Catch does not differ significantly from other treatments with beetles ($P>0.05$, $\alpha=0.007$)
[e] Catch differs significantly from others ($P<0.002$, $\alpha=0.006$)
[f] Catch does not differ significantly from that on log ($P>0.1$, $\alpha=0.006$). In the above tests of statistical significance (c–f), the Wilcoxon test was used to compare treatments as indicated at $\alpha=0.05$/ number of comparisons

compete for the limited number of wind-thrown and less resistant pines in which the beetle almost exclusively breeds [7]. The vagaries of spring weather also necessitates that beetles locate suitable breeding areas in as short a period as possible – one or two days. Their mass aggregation in response to the monoterpenes that are released from lacerations on storm-injured and fallen trees (415 beetles
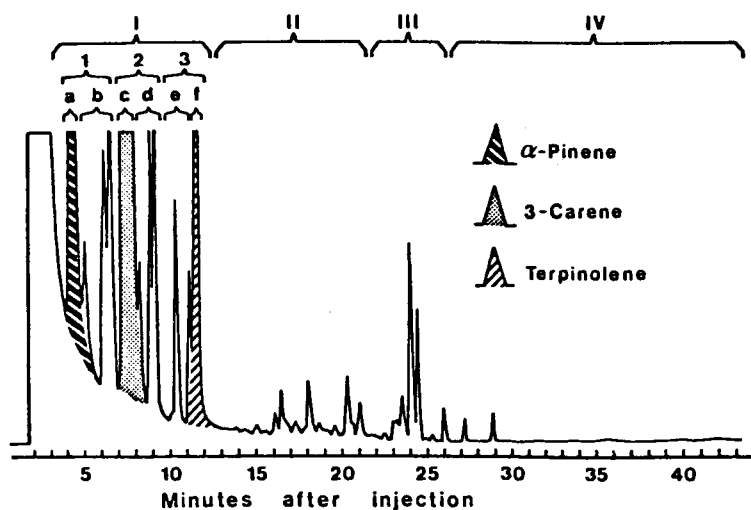

Fig. 1. Gas chromatogram (FID) of volatiles from a 30 male- and 30 female-infested Scots pine log (25 cm × 13 cm diam.). Numerals, numbers and letters above peaks indicate the collection periods from GC column effluent during separation into various fractions with a manually rotated fraction collector containing six flow-through glass tubes cooled to −78 °C. The GC glass-capillary column (35 m × 0.5 mm i.d.) was coated with a film of 1.3 μm OV-351 (Supelco) and temperature programmed at 60 °C for 5 min, then 5 °C/min to 230 °C and then isothermal at 20 cm^3/s N$_2$ flow. The three major peaks as indicated, which had behavioural activity, were identified by matching their retention time and mass spectra with that of authentic standards on a Finnigan 4021 gas chromatograph-mass spectrometer (GC-MS). For the GC-MS analyses a fused silica-capillary column (25 m × 0.2 mm i.d.) of Superox FA (Alltech. Assoc.) of 0.4 μm film was used at similar temperature/ He flow program as above

Table 2. Attraction response of walking male and female *Tomicus piniperda* to monoterpenes occurring in Scots pine (28 April–8 May 1983). Monoterpenes were diluted in diethyl ether and released from 5 μl capillary tubes in a laminar air flow in a laboratory olfactometer [3] under dim red light at 8 lux

Test solution	Percent responding – males/females		
	Amounts released (× 2.2 g compound/min)		
	10^{-7}	10^{-6}	10^{-5}
(+)-3-Carene	23/17	40[a]/47[a]	50[a]/40[a]
(+)-α-Pinene	20/23	33[a]/53[a]	47[a]/47[a]
(−)-α-Pinene	23/30[a]	50[a]/37[a]	50[a]/47[a]
Terpinolene	27[a]/30[a]	53[a]/57[a]	53[a]/60[a]
Myrcene	10/17	10/7	10/17
Ether control	10/13	3/7	10/10

[a] Value ($N=30$) differs significantly from diethyl ether control ($N=90$) ($P<0.01$, χ^2)

were caught on the two uninfested logs on 27 March 1982) would provide a mechanism for recognizing this suscep-

tible host material as well as locating mates soon after taking flight. Healthy resistant trees are not attractive [7] since there are no wounds exuding oleoresin and so they presumably are releasing subliminal amounts of the monoterpenes.

We found no evidence that *T. piniperda* uses a long-range attraction pheromone as shown for practically all other temperate bark beetles that have been studied [1]. This could be because the direct attraction to host monoterpenes immediately indicates a damaged and less resistant host at this time of year. Thus it is a more efficient mechanism for *T. piniperda* compared with a pheromonal system of aggregation, in which more time would be required for production and release from an exponentially growing colony. The three monoterpenes are major components of Scots pine oleoresin (Fig. 1) but are found in lower amounts or not at all in Norway spruce [8], the other predominant conifer in Northern Europe. This appears to explain in part the beetle's host-species selection of Scots pine. The pine shoot beetle's olfactory

recognition of host-species and host-susceptibility that leads to mating is a parsimonious, rapid and energy-efficient mechanism resulting in higher reproductive benefits.

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