Host tree unsuitability recognized by pine shoot beetles in flight

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Summary. In spring, the landing rate of flying European pine shoot beetles, *Tomicus piniperda* L., on injured Scots pine diminishes as colonization continues. This is due to olfactory cues that indicate progressive host degradation. Verbenone was shown to play a role in the beetle's recognition of this unsuitability of a formerly suitable host, since the compound was increasingly released from colonized tree sections as they aged, but not from uninfested sections. Also, the release of verbenone at natural rates in the forest inhibited the attraction of beetles to host monoterpenes.
Key words. Semiochemical; verbenone; *Tomicus piniperda*; Scolytidae; bark beetle; *Pinus sylvestris*; host selection.

The question of how insects select their host plants is one of the important areas of investigation into insect herbivory[1]. A related topic which has been much less investigated is how insects may recognize the relative suitability of a food resource which has become increasingly unsuitable due to competition from established conspecifics, or to general microbial degradation leading to a decrease of food quality[2]. Several theories on how bark beetles terminate their aggregation concern olfactory mechanisms which are based on observations of a reduced response to attractants by a specific inhibitor associated with the beetle[3-5]. However, the rates of release are not known for either the natural inhibitor or for the synthetic inhibitor, or both[3-5]. Thus, the theories remain unproven. In this study, we attempt to quantify the relationship between the attractant and inhibitory semiochemicals in the natural system so that they can be tested experimentally at a natural release rate.

Pine shoot beetles aggregate in large numbers on fallen Scots pine within tens of minutes after the temperature rises above 12 °C on the first swarming day of spring[6,7]. The species is unusual compared to other aggregating bark beetles in that it does not appear to use a long-range pheromone but instead is strongly attracted to volatiles from host logs[6]. At least three monoterpenes are responsible for this attraction to the host, (+/−)-α-pinene, (+)-3-carene and terpinolene, which are released in relatively large quantities from wound oleoresin exudations[6]. This olfactory mechanism allows the beetle both to select the host tree from among non-hosts and to recognize an injured tree as being especially susceptible to attack colonization, due to a lower resinous exudation capacity as a result of storm damage[6].

In the earlier stages of aggregation when the densities of attack are lower, there are few adverse effects of competition[9,10], but as the density of colonization increases over time, the detrimental effects of competition become increasingly apparent in the reduced brood output per female[10,11]. This relationship has been found in several species[11] and is a logical expectation of ecological theory. What is poorly understood, however, is the qualitative and quantitative nature of the olfactory mechanism that plays a role in terminating the aggregation due to the individual's avoidance of fully colonized areas.

To determine whether *T. piniperda* beetles recognize when a host tree becomes less suitable as colonization progresses, we followed the relative attractiveness of colonized trees through time compared with traps releasing constant rates of monoterpenes (fig. 1). Twenty flight-barrier traps, consisting of a transparent plastic pane (17 × 35 cm high) with funnel and collection bottle, were placed next to six Scots pines, *Pinus sylvestris* (L.), which had fallen in winter storms and had subsequently been colonized by *T. piniperda*. The barrier traps were sampled daily (April 18–May 6, 1986) and the catches of beetles were compared to catches on four perforated cylinder

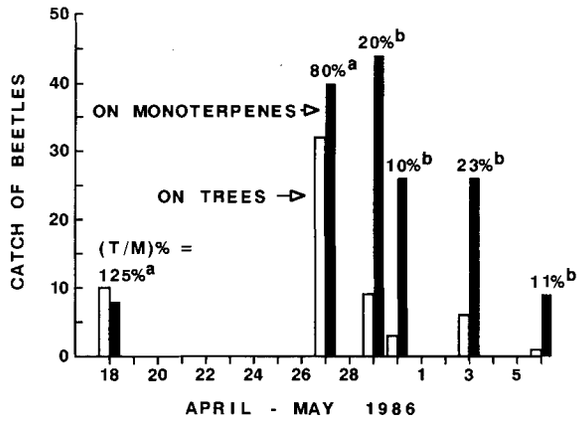

Figure 1. Relative attraction rates of *Tomicus piniperda* to six winter-storm-fallen Scots pine trees with twenty flight-barrier traps and to constant release rates of monoterpenes from four perforated cylinder traps during the annual spring colonization period (Sjöbo, southern Sweden). The percentage of catch (based on total for a trap type; trees/monoterpenes = T/M %) between dates were significantly different ($\alpha = 0.05$, x^2) if designated with different letters. Each cylinder trap[8] released (per day) a mixture of monoterpenes (14 mg each (+)-α-pinene, > 99% pure, $[\alpha]_{546}^{20} = +57°$, and (−)-α-pinene, > 99.5%, $[\alpha]_{546}^{20} = -50°$; 6 mg (+)-3-carene, > 99%, $[\alpha]_{D}^{20} = +17°$, all Fluka; and 2.5 mg terpinolene, > 97.3%, Carl Roth) as described in the table.

traps[8] baited with the three attractive host monoterpenes released at constant rates[6] (fig. 1). Attacks of *T. piniperda* on the trees were also observed beginning on April 17, and most attacks had occurred by April 19 (mean 2.25/dm^2, n = 6) since cool weather then precluded flight and few additional attacks occurred after April 28 (mean 2.85/dm^2, n = 6).

The daily catches on the traps releasing constant rates of monoterpenes presumably reflect the population density of flying beetles. These catches can then be compared to the corresponding catches of the barrier traps on the fallen trees, to obtain an estimate of the relative attractivity of infested trees during the colonization period (fig. 1). It can be seen that early in the colonization there was a relatively high attraction to the host trees (catching 125% of that of the monoterpene traps on April 18), but as the trees were progressively colonized during the spring flight period, there was a relative decline in the attractivity of the colonized hosts (fig. 1). This decline in attractivity indicates that flying beetles avoid or do not respond to hosts when they become increasingly more unsuitable due to food resource utilization and/or potential competition from established conspecifics[9–11].

Hindguts of bark beetles may contain pheromone components that promote or inhibit attraction[3–8], although we are not certain what this means in the natural context. In *T. piniperda* feeding in host tissue, the hindguts of both sexes contain verbenone among other constituents[7]. Until this study was carried out, verbenone had not been tested for behavioral activity, but it had been shown to elicit the highest electroantennogram responses from among several hindgut volatiles of *T. piniperda*[7]. Verbenone was shown to inhibit the attraction response to pheromone of some closely related *Dendroctonus*[12–15] as well as *Ips species*[16,17], although nothing was known about natural release rates. Therefore, we investigated whether verbenone, tested over a wide range of release rates, could reduce the attraction response of *T. piniperda* to the host monoterpenes in a laboratory bioassay. Walking beetles were tested in an open arena (46 cm diameter) for their upwind orientation to semiochemicals released from diethyl ether solvent in a 5-µl glass capillary[7]. The tests showed that the attraction of both sexes to a constant release of host monoterpenes was significantly reduced as the release of (−)-verbenone was increased (fig. 2).

To quantify the release rate of verbenone from infested host substrates, odors were collected from air passed at 90 ml/min over host logs, with or without beetles, (28 cm × 14 cm diameter) in glass containers (30 cm × 16 cm diameter). Each log had 50, 3-mm drilled holes and was uninfested, or was infested first with 50 females and then 2 h later with 50 males. The odors in the air effluent from each container were adsorbed on Porapak Q plugs (55 × 7-mm diameter of 80/100 mesh, Supelco Inc.), extracted every 12 or 24 h. Volatiles were eluted from the plugs with 3–4 ml pentane/diethyl ether (9:1) and separated on a 20 mm × 5 mm i.d. activated silica column (Silic AR, Malencrodt) by stepwise elution with the above solvent and then diethyl ether. Volatiles were analyzed for monoterpenes and oxygenated derivatives (e.g. verbenone and *trans*-verbenol) by capillary gas-chromatography and mass spectrometry (GC-MS).

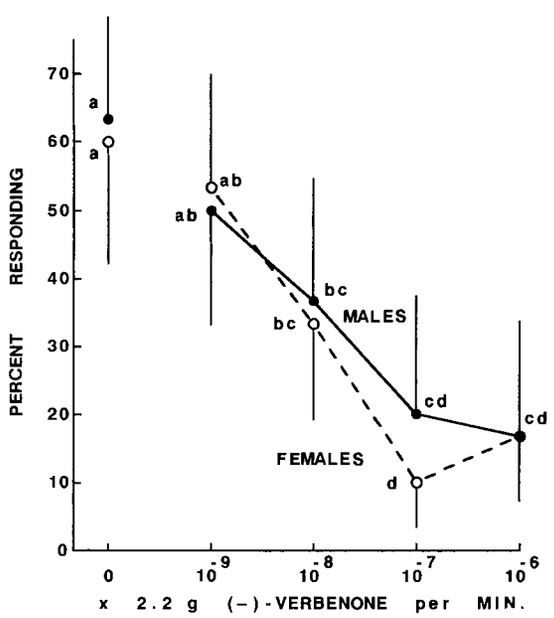

Figure 2. Effect of increasing release rates of (−)-verbenone on the attraction response of walking male and female *Tomicus piniperda* to a 1:1:1:1 mixture of host monoterpenes (−)-α-pinene, (+)-α-pinene, (+)-3-carene and terpinolene (chemical purity as in fig. 1, table) each released at 2.2×10^{-6} g/min. Points (N = 30) with the same letters were not significantly different ($\alpha = 0.05$, x^2). The vertical lines represent upper or lower 95% confidence limits for proportions.

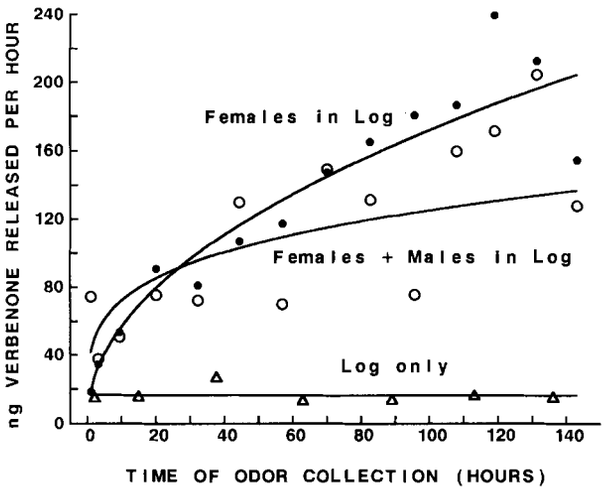

Figure 3. Release of verbenone from Scots pine logs infested with 50 female (filled circles), or 50 female plus 50 male (open circles) *Tomicus piniperda*, or not infested but drilled with 50 holes (triangles). Lines were fitted with geometric curve regression, $Y = 18.93X^{0.479}$ (females in log), $Y = 41.73X^{0.238}$ (females + males in log) and $Y = 16.94X^{-0.008}$ (log only).

Quantification was done by reconstructed ion chromatogram comparisons to authentic standards and using *Z*7-tridecenyl acetate (500 ng) as internal standard on a Finnigan 4021 GC-MS and capillary column (25 m × 0.15 mm i.d. fused silica and Superox FA 0.37 µm film temperature programmed for 5 min at 60 °C, then 8 °C/min to constant 220 °C).

The amount of verbenone released from infested logs began to increase geometrically after the introduction of females ($r^2 = 0.97$), and during the 6-day period rose to about 12 times more from the female-infested log than from the control log, from which release remained constant (fig. 3). The geometric relationship for verbenone release from infested logs does not coincide well with the amounts of verbenone in the hindguts of both sexes; these contain the largest amounts (up to 4 ng/individual) when the mating chamber is being constructed, during the first 24–48 h, and then amounts decline [7]. In contrast to verbenone, a quadratic relationship was observed for *trans*-verbenol with a peak release (602 ng/h) at 85 h for the female-infested log ($r^2 = 0.82$) and at 58 h for the male + female-infested log (277 ng/h), and then amounts declined. Only constant low amounts of *trans*-verbenol release were found for the control log (about 31 ng/h). The pattern of *trans*-verbenol release is better correlated with amounts found in hindguts during the same period of time [7]. However, it is not possible to determine what proportion of the verbenone (or *trans*-verbenol) we collected is derived from beetles and how much is from other sources, since the excretion rates of semiochemical from beetles are not known.

An alternative hypothesis is that microorganisms introduced by the beetles populated the host tissue and became the more significant producers of verbenone in the later stages of colonization. In other bark beetles, certain associated fungi (yeast phase) can convert *trans*-verbenol, present as an oxidation product of α-pinene, into verbenone in culture media [18, 19]. However, it has not been shown that these fungi are responsible for verbenone production under natural conditions or that they are associated with *T. piniperda*.

The release rates of α-pinene and 3-carene from the infested logs (14 µg/h and 13 µg/h, respectively) were determined during the last half of the six-day collection period using GC-MS in order to determine a natural release rate of host monoterpenes relative to verbenone for use in field experiments. The perforated cylinder traps were used in pine forest to compare an attractive release rate of the three host monoterpenes to similar releases in combination with releases of either (+)- or (−)-verbenone (table). Both enantiomers of verbenone inhibited attraction to host monoterpenes (table), although only the (−)-enantiomer was highly pure (99.2% e.e.) and is clearly active alone. Therefore, there is no evidence of synergism between the enantiomers in eliciting the activity [20].

Based on the odor collections, the release rate in the forest of the attractive monoterpenes, α-pinene and 3-carene, and the inhibitor verbenone were equivalent to the amount released from about five large logs (1 m × 40 cm diam.). Natural release rates would differ somewhat from this estimate, depending on the age of the infested material. The decline in attractiveness of the fallen trees (fig. 1) appears to be due in large part to an increasing verbenone release (fig. 3) and possibly in small part to a decline in monoterpene release. The decline in monoterpenes from the infested test logs was about 50% over the 6-day period, but release from larger diameter logs may decline more gradually. In the laboratory

Attraction of *Tomicus piniperda* to perforated cylinder traps [8] releasing attractive host monoterpenes and inhibitory enantiomers of verbenone (V) in Scots pine forest, Sjöbo, southern Sweden. *T. piniperda* beetles were caught in the outer funnel (while landing) and only one entered a hole in the perforated cylinder.

Chemicals released [a]	Total catch of cylinder traps	
	Males	Females
14–20 Mai 1985 (9 replicates)		
Blank	0	0
Monoterpenes [b]	30	29
Monoterpenes + (+)-V	9	3
Monoterpenes + (−)-V	0	3
17–29 April 1986 (13 replicates)		
Blank	1	0
Monoterpenes [b]	83	65
Monoterpenes + (+)-V	35	16
Monoterpenes + (−)-V	19	19

[a] Chemicals were each released at constant rates from two tubes (3 cm × 0.6 cm diameter) each containing 0.1 ml compound (neat) from inside the cylinders as in fig. 1. Verbenone (V) enantiomers were each released as above but at 0.25 mg/day (in 1985: 85% pure, 90% e.e., Borregaard, in 1986: (−)-V was > 99% pure, $[\alpha]_D^{20} = -246°$, 99.2% e.e., Bedoukian). [b] Wilcoxon tests indicated that the monoterpene-releasing traps caught significantly more beetles than either the blank traps or similar traps also releasing verbenone enantiomers ($p < 0.05$).

bioassay (fig. 2), significant behavioral effects brought about by verbenone occurred at ratios from 1:100 to 1:1 (verbenone:monoterpenes), or in absolute amounts equivalent to one large log (for monoterpenes) and 0.6 to 63 large logs (for verbenone).

Several earlier studies have shown that verbenone (natural release unknown) could inhibit the attraction response of bark beetles to pheromone[12-17]. Based on these results, a theory was proposed for the western and southern pine beetles, *D. brevicomis* and *D. frontalis*, that males would join females and release verbenone to cause the termination of aggregation[4]. In none of these studies, however, have naturally infested host substrates been compared to constant release rates of attractant chemicals to determine the rate of decline in attraction to colonized hosts as they age. Also, no earlier study has quantified the release of verbenone over the colonization period to determine whether it could account for termination. One other study did measure the release of verbenone from infested substrates, but only for the first day of colonization, and no conclusions were drawn[21].

The spatial attack pattern of *T. piniperda* on the trunk of a Scots pine was shown to be more uniform than random by nearest neighbor analysis[22]. Thus, *T. piniperda* females avoid initiating an attack that is too close to others, and this spacing mechanism could be the result, in part, of an avoidance of verbenone (as shown in the walking bioassay, fig. 2). We have shown that verbenone is released in increasing amounts following the initial stages of colonization. Natural selection would favor the evolution of bark beetles which could recognize volatile chemicals that were consistently associated with degrading and/or fully-colonized trees so that these could be avoided. Once a predominant bark beetle species utilized verbenone as a chemical signal of host unsuitability because of intraspecific colonization, then other sympatric species might have come to rely on verbenone to avoid interspecific competition as well as to avoid host-plants that are unsuitable because of degradation and/or conspecific colonization. For instance, *I. paraconfusus* avoids logs infested with *D. brevicomis* (males contain verbenone) and verbenone in California, where the species compete for the phloem food of ponderosa pine[16]. Further chemical and behavioral studies with airborne collection are needed because only a few studies have quantified the volatile effluents of moths[23] and bark beetles[21,24] over short periods. Quantitative measurement of airborne release of semiochemicals over a several-day period of colonization in combination with quantitative estimates of behavioral response in the laboratory and in the field provide a basis for understanding the olfactory mechanisms that insects use to recognize their host and its condition. Recently we have shown that (–)-verbenone protects Scots pine logs from attack by *T. piniperda* in the forest[25].

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