

tion benzene was not considered, because the method applied did not allow its quantitative determination. The use of modified methods suitable for also detecting low-boiling organic compounds made it possible to obtain vertical concentration profiles for this class of compounds, too. The close relationship shown by the seasonal and spatial distribution of both compounds, benzene and toluene, points to a common biogenic source. While different aromatic compounds introduced into the water body by pollution or runoff from the lithosphere (C₂- and C₃-benzenes) exhibited fairly closely related concentrations in both the oxygen-containing and oxygen-depleted water layers, the concentrations of benzene and toluene increased significantly towards the sediment. After the turnover of the lake water, a homogeneous distribution of both compounds was observed in the lake. As soon as an anoxic hypolimnion developed after stratification of the lake, the benzene and toluene concentrations again increased in the hypolimnion. Intense degradation of organic matter and protein as

indicated by the high concentrations of ammonium ions present was taking place. Nitrate as a potential precursor for ammonium formation can be excluded since the input of oxidized nitrogen into the lake is rather low. Further evidence of the close relationship between protein degradation and the formation of benzene and toluene can be found in the concomitant occurrence of high concentrations of p/m-cresol (which isomers were not separated but have been shown to be p-cresol in an investigation the year before [7]) that is restricted to the zone near to the bottom of the lake. The accumulation of benzene and toluene in the anoxic hypolimnion also suggests that degradation processes of aromatic compounds which occur under denitrifying conditions [8] or which have been shown for methanogenic consortia [9] could not compete with the production.

Reports on a biogenic formation of benzene from carbon compounds such as phenylalanine are lacking and may have been overlooked. The data of Lake Schleinsee, however, indicate that

such processes may occur in anoxic environments.

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Synergistic Pheromones and Monoterpenes Enable Aggregation and Host Recognition by a Bark Beetle

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We report the strong synergism between two pheromone components in causing the attraction of the six-spined spruce bark beetle or "Kupferstecher", *Pityogenes chalcographus* L., in the field. Our field results also suggest that several monoterpenes of Norway spruce, *Picea abies* (L.) Karst, when presented with the pheromone components, play a role in host recognition

and colonization by the beetle because they caused relatively more beetles to enter holes in artificial hosts. Furthermore, sexual differences in strategies of host resource competition are indicated because of the lower proportions of males than females that entered the holes as well as were attracted to higher release rates of pheromone.

In Europe, *P. chalcographus* is a serious pest of Norway spruce upon which the tiny adults (2 mm long) aggregate in response to a male-produced pheromone that earlier was reported to

consist of at least one component, chalcogran, a unique dioxaspiro acetal [1]. However, the release of 15 mg h⁻¹ of synthetic chalcogran was not as attractive as a host log infested with 30 males, suggesting that a second component may be necessary for maximum response [1]. Recently, we used two-dimensional gas chromatography and subtractive-combination bioassay to isolate a second male-produced component, methyl-*E,Z*-2,4-decadienoate (*E,Z*-MD), from odors of beetle-infested logs [2]. *E,Z*-MD and chalcogran were further shown to be synergistically attractive in the laboratory bioassay, while an additional several monoterpenes in mixture caused a slight enhancement of the response [2].

We tested *E,Z*-MD and chalcogran for their attractivities alone and in combination in the forest during the spring dispersal/host-seeking flight of *P. chalcographus* (Table 1). Traps with the pheromone components or blanks were placed 10 m apart in a line in clear-cut

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areas of Norway spruce forest. Each trap consisted of a vertically oriented black plastic cylinder (1.35 m × 12 cm diam. with 900, 2-mm diam. holes uniformly spaced over the surface [3]) that was centered over a 33-cm diam. funnel. Beetles were collected either during landing by this funnel (by striking the cylinder and falling or by flying downward) or after they landed and entered one of the holes in the cylinder by a smaller 12-cm diam. funnel fixed at the

bottom (by slipping inside the cylinder).

In test 1 (Table 1), the combination of the pheromone components was clearly synergistic, catching 34 times more than chalcogran, while *E,Z*-MD had no activity alone (19 replicates, Wilcoxon $P < 0.01$). In the second test (Table 1), a mixture of several host monoterpenes that were released with the pheromone components caused a significant increase in the proportion of both sexes

that entered holes in the cylinder trap. Each of the various monoterpenes when released with the pheromone components also caused a greater percentage of both sexes to enter holes (test 3, Table 1). These results indicate that the monoterpenes, especially a mixture, when released from a 12-cm diam. cylinder can simulate a host tree and induce hole-entering and thigmotactic behavior in both sexes. (\pm)- α -Pinene, (-)- β -pinene, and camphene, major volatiles of host oleoresin [2], are thus used by *P. chalcographus* to recognize its host during colonization. The release rates used here are comparable to those released from infested host material [2].

If possible, it would be to the advantage of a male to enter holes releasing monoterpene vapors because this would indicate a direct route to the phloem tissue without the necessary energy and time expenditure of boring through the outer bark. On the other hand, females normally enter holes with monoterpenes and pheromone component vapors because this indicates the host tissue needed for egg laying as well as a male for mating and possible brood protection (the male guards the entrance to the gallery system). Monoterpenes either alone [4] or usually in combination with pheromone components [5] have been shown to be attractive to bark beetles, but the effect on hole-entering has not been reported earlier.

Increasing release rates over a 100-fold range of *E,Z*-MD and chalcogran were tested to observe response relationships in both sexes (test 4, Table 1). Catch increased in a logarithmic relationship, $Y[\%] = 66.88 + 16.39 \times \ln X$ (where $X = 0.1$ to 10 and maximum catch = 100%), but the highest release rate may have caused some disorientation (confusion) near the source because the increase in catch was proportionately less than the increase between the lowest and middle doses. While there was no apparent effect of release rate on the percentage entering holes, there was an effect on the male proportions that landed or entered holes.

The proportion of males in the landing catch decreased as the release rate was increased (test 4, Table 1). This effect of relative male inhibition by higher releases of pheromone has also been observed in other polygynous bark

Table 1. Attraction of *Pityogenes chalcographus* to perforated cylinder traps releasing synthetic host ((+)- α -pinene = (+A), (-)- α -pinene = (-A), (-)- β -pinene = (-B), and camphene = Cp) and pheromone components (methyl-*E,Z*-2,4-decadienoate = *E,Z*-MD and chalcogran = CH) in the forest (June 1–15, 1986, Grib skov, Denmark)

Chemicals released ^a	Attraction to cylinder trap				
	Landing		Entering holes		[%]
	Total	Male proportion	Total	Male proportion	
		test 1			
Blank	1	—	0	—	0
CH	39	0.26	8	—	17.0
<i>E,Z</i> -MD	0	—	0	—	—
<i>E,Z</i> -MD + CH	1376	0.42	240	0.35	14.9
		test 2			
<i>E,Z</i> -MD + CH	123	0.46	7	—	5.4 ^b
<i>E,Z</i> -MD + CH + (+A) + (-A) + (-B) + Cp	482	0.40	338	0.35	41.2
		test 3 ^c			
<i>E,Z</i> -MD + CH	763	0.33	103	0.32	11.9 ^b
<i>E,Z</i> -MD + CH + (+A)	720	0.33	318	0.29	30.6
<i>E,Z</i> -MD + CH + (-A)	1476	0.33	815	0.28 ^d	35.6
<i>E,Z</i> -MD + CH + (-B)	1077	0.35	618	0.27 ^d	36.5
<i>E,Z</i> -MD + CH + Cp	749	0.33	208	0.24 ^d	21.7
		test 4			
Blank	4	—	0	—	0
0.1 × (<i>E,Z</i> -MD + CH)	665	0.43 ^c	139	0.26	17.3
1 × (<i>E,Z</i> -MD + CH)	1968	0.37	534	0.33	21.3
10 × (<i>E,Z</i> -MD + CH)	2772	0.34	507	0.30	15.5

^a Except as indicated in test 4, chemicals inside each cylinder were released (per day) at 18 μ g *E,Z*-MD, >99.5% GC pure; 1 mg CH, 46% *E*:54% *Z*, >98% pure from W. Francke, Univ. of Hamburg, West Germany; 14 mg (+A), $[\alpha]_D^{22} = +48^\circ$; 14 mg (-A), $[\alpha]_D^{22} = -42^\circ$; 10 mg (-B), $[\alpha]_D^{22} = -21^\circ$; and 40 mg Cp (all monoterpenes >98% pure, Fluka and Carl Roth).

^b Percentages of males or females entering holes were significantly different from those entering treatments releasing monoterpenes ($P < 0.001$, χ^2 , except for males to Cp, $P = 0.11$).

^c Wilcoxon tests indicated the catch was significantly greater on pheromone (*E,Z*-MD + CH) plus (-A) baits than on pheromone alone ($N = 10$, $P < 0.01$).

^d Male proportions were significantly different from corresponding proportions of landing ($P < 0.01$, χ^2).

^e Male proportion was significantly different from male proportion entering holes or from male proportions of landing at the higher release rates ($P < 0.01$, χ^2).

beetles, *Ips paraconfusus* [6] and *I. ty-pographus* [7]. Furthermore, the relative proportion of males caught decreases between landing and entering holes in all cases either with or without monoterpenes, and at all release rates (Table I). In several cases, the differences were significant ($P < 0.01$, χ^2), as were the differences between the pooled results for the male proportions of landing (0.384) and entering (0.335) in response to the pheromone components alone ($P < 0.01$) as well as between the male proportions of landing (0.340) and entering (0.286) in response to the pheromone with monoterpenes ($P < 0.001$). This again indicates that males may be proportionately less attracted by higher release rates encountered while proceeding up the pheromone concentration gradient. It has been proposed that males of the polygynous bark beetle species (e.g., *Ips*, *Pityogenes*) which produce the pheromone and establish territories of resource utilization will likely be relatively less attracted by higher release rates of their pheromone than are

females [6]. Higher release rates would indicate to arriving males that the emanating areas are fully colonized and are thus to be avoided in order to avoid competition for food and space resources. Females, on the other hand, require male-initiated galleries and thus the male-produced aggregation pheromone serves additionally as a sex pheromone.

The highly synergistic properties of *E,Z*-MD and chalcogran in attracting *P. chalcographus* indicate that a potent pheromone blend has been identified. Host monoterpenes may enhance slightly the attraction and further serve to increase catch through hole-entering in certain kinds of traps. Bark beetle control methods using pheromone-baited traps have recently been undertaken [8]. This method removes the most vigorous beetles, those that are flying and responding and are potentially the most damaging of the population. Trap-out methods are selective, environmentally safe, and do not compete significantly with other natural mortality agents during the dispersal

flight when probably most adult mortality occurs. Based on our results *E,Z*-MD and chalcogran have recently been marketed as a commercial lure ("Chalcoprax", Shell Agrar GmbH & Co. KG, P.O. Box 200, D-6507 Ingelheim am Rhein) for trapping of *P. chalcographus* [9].

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