*chelys aprix* from the Early Jurassic of North America [11], also possesses four pairs of pores on inframarginal scales (Fig. 2). These pores in *K. aprix* establish Rathke's gland as the oldest amniote skin gland.

We are unable to document Rathke's gland pores in several extinct taxa, including the oldest pleurodire, *Proterochersis robusta* from the Late Triassic of Germany [12], and a variety of cryptodire groups (Fig. 1). Neither do we observe gland pores in the most primitive turtle, *Proganochelys quenstedti* from the Late Triassic of Germany [13]. Aside from the obscurity of gland pores, determining the presence of Rathke's gland in some extinct taxa is problematic because the developed skeletal components through which gland ducts would otherwise pass en route to the shell bridge or skin are lacking.

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# Volatiles from Nonhost Birch Trees Inhibit Pheromone Response in Spruce Bark Beetles

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Volatiles from nonhost birch bark, *Betula pendula*, inhibit the orientation response of bark beetles, *Pityogenes chalcographus* and *Ips typographus* (Coleoptera: Scolytidae) to their aggregation pheromone components. Odors from leaves of *B. pubescens* also reduce attraction of both species. Neither species colonized birch trees baited with synthetic pheromones while readily accepting their host Norway spruce, *Picea abies*. (*Z*)-3-Hexen-1-ol and 1-hexanol were found in air drawn from birch leaves, and both compounds reduced attraction of these beetles to their pheromone. Bark beetles may have evolved these behaviors to avoid wasting time investigating or boring in nonhost trees.

The bark beetles *I. typographus* and *P. chalcographus* are important enemies of Norway spruce, *P. abies*, the dominant tree of northern European forests (Austarå et al. 1984). These beetles must find a susceptible host tree and then aggregate in large numbers by attraction to a pheromone in order to overpower the resinous defenses of the tree (Byers 1995, 1996). The host-finding phase of bark beetles is the most risky period of the life cycle, with mortality up to 80% or more as judged by ratios of emergence to entrance hole numbers in bark samples (Byers 1996). Thus it would be advantageous for bark beetles to find their host tree as quickly as possible. Natural selection may have caused bark beetles to evolve several mechanisms for finding their host tree as well as for avoiding unsuitable host and nonhost species of plants. Host selection has long been hypothesized to be a complex process comprising both affinity for host chemicals and avoidance of nonhost chemicals (Thorsteinson 1960).

Many of the most "aggressive" tree-killing bark beetles are believed to find host trees initially by landing at random since there is no evidence of a long-range attraction to uncolonized hosts (Moeck et al. 1981; Byers 1995, 1996). Most of these bark beetle species exhibit reduced attraction to

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pheromone in the presence of unsuitable hosts that are decayed or fully colonized and releasing ethanol and verbenone (Bakke 1981; Klimetzek et al. 1986; Byers et al. 1989; Schlyter et al. 1989; Schroeder and Lindelöw 1989; Byers 1992, 1993, 1995). In addition, studies with the North American species *Dendroctonus frontalis*, *I. grandicollis* and *I. avulsus* indicate that bark beetles may avoid nonhost trees because their response to pheromone is inhibited by several compounds among the "green-leaf volatiles" (Dickens et al. 1991, 1992). In Europe the weak attraction to ethanol, believed released from damaged Scots pine hosts, by *Tomicus piniperda* (Schroeder and Lindelöw 1989; Byers 1992) has been shown to be reduced by odors from logs of nonhosts aspen (*Populus tremula*) and birch (*B. pendula*; Schroeder 1992). Subsequently the aggregation responses of *I. typographus* and *I. duplicatus* to pheromone and *T. piniperda* to host monoterpenes have been shown to be inhibited by a blend of six green-leaf volatiles (Schlyter et al. 1995). Recently the responses of North American *D. ponderosae* and *Trypodendron lineatum* to pheromone components have been shown to be reduced by (*Z*)-3-hexen-1-ol and other green-leaf volatiles (Wilson et al. 1996; Borden et al. 1997).

Two species of birch, *B. pendula* and *B. pubescens*, are the most common deciduous trees in Norway spruce forests of Scandinavia. To determine whether spruce bark beetles, *I. typographus* and *P. chalcographus*, avoid the nonhost birch, pheromone baits were placed in a trap-pair rotor that separated the baits by 6 m at 1.2 m height and revolved at 2 rph (Byers et al. 1990). The traps consisted of plastic cylinders (18 cm diameter, 28 cm high) covered at the top but open at the bottom and suspended over a funnel (31 cm diameter) that collected beetles striking the cylinder (Fig. 1). Appropriate pheromone baits (Bakke et al. 1977; Francke et al. 1977; Byers et al. 1988, 1990) were placed inside the cylinders that also contained either a fine screen cage or one containing freshly cut birch bark chips or leaves (Fig. 1). (*Z*)-3-Hexen-1-ol was tested similarly with pheromone baits since it is a known volatile from birch, *B. pendula* (König et al. 1995), and is also active in reducing aggregation of *D. ponderosa* and *T. lineatum* in North America (Wilson et al. 1996; Borden et al. 1997). 1-Hexanol was tested because it disrupts pheromone response in other bark beetles (Dickens et al. 1991, 1992) as well as being found earlier in hindguts of male *P. chalcographus* (Francke et al. 1977).

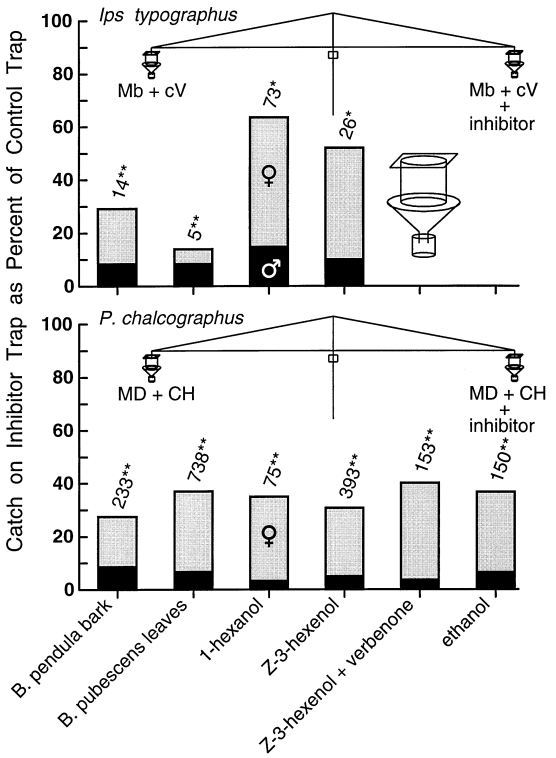


Fig. 1. Reduction in catch of *Ips typographus* and *Pityogenes chalcographus* on traps releasing pheromone plus inhibitor volatiles compared to a control pheromone trap. The trap pairs were rotated mechanically at 2 rph to minimize catch variation due to trap position. Test replicates were conducted for at least 1 h, and after each replicate the inhibitory source, but not the attractants, was switched to the other trap. Replicate catches were summed, and the paired control and treatment were compared by a $\chi^2$ goodness of fit test to an expected catch if there are no differences based on the average for the two traps. About 100 g leaves and 400 g bark chips were used in various tests during June (1997 and 1998), Torsby, Sweden. *I. typographus* pheromone components 2-methyl-3-butenol (*MB*) and *cis*-verbenol (cV), were released at 50 mg and 1 mg/day, respectively. (*Z*)-3-Hexen-1-ol and 1-hexanol (both 98%, Aldrich), verbenone ($[\alpha]^{20}D = -246°$, 99.2% e.e., Bedoukian), and ethanol were released at 8.6, 5.7, 1, and 800 mg/day, respectively. *P. chalcographus* pheromone components, *E*,*Z*-2,4-methyl decadienoate (*MD*, >99.5%) and chalcogran (*CH*, 46% E:54% Z, >98%), were released at 70 μg and 0.8 mg per day, respectively. $^*P < 0.01$, $^{**}P < 0.001$ catches (*numbers above bars*) were significantly different between baits in the same test ($\chi^2$ goodness of fit)

The tests showed that volatiles from birch bark and leaves inhibited the attraction of both *I. typographus* and *P. chalcographus* to their synthetic pheromones (Fig. 1). The green-leaf volatiles, (*Z*)-3-hexen-1-ol and 1-hexanol, also reduced attraction in both bark beetle species (Fig. 1). Males of *P. chalcographus* were inhibited more so than females by odors from birch leaves or the two green-leaf volatiles since male percentages of catch (9–17.5%) were significantly lower than on the pheromone controls (19.5–26%; $P < 0.001$ in all cases). No differences in catch between the sexes were observed due to bark volatiles or in any comparison with *I. typographus*.

The addition of verbenone, a known inhibitor of *P. chalcographus* (Byers

1993), further reduced the inhibition of *P. chalcographus* by (*Z*)-3-hexen-1-ol (Fig. 1), indicating a synergism between the two inhibitors. Verbenone plus a blend of six green-leaf compounds has earlier been indicated as being synergistic in reducing response in *T. piniperda* and *I. typographus* (Schlyter et al. 1995). Ethanol at 800 mg/day, a relatively high release rate (Schroeder and Lindelöw 1989; Byers 1992), reduced the attraction of *P. chalcographus* to pheromone (Fig. 1). This is in accordance with the theory that ethanol from decadent hosts reduces response of "primary" bark beetles such as *I. typographus* (Klimetzek et al. 1986) that colonize living trees releasing little or no ethanol (Moeck 1970; Kimmerer and Kozlowski 1982).

To determine whether (*Z*)-3-hexen-1-ol, 1-hexanol or other compounds are present in odor from birch, volatiles were collected from bark of *B. pendula* and shoots with leaves of *B. pubescens* from the same trees as used in the field experiments, as well as from leaves of both species from Lomma (near Malmö), Sweden. The uncut ends of the shoots with leaves or bark chips were enclosed in plastic cooking bags (Meny® Toppits®, 35 × 43 cm) through which activated-carbon filtered air was sucked at 150 ml/min. The effluent volatiles were adsorbed on 30 mg Porapak Q (50–80 mesh, Supelco) in a 3-mm ID Teflon tube for 2.5 h. Diethyl ether washings (300 µl) of the Porapak Q were kept at –20° C until chemical analysis on a combined HP 5890 series II gas chromatograph and HP 5972 mass selective detector (GC-MSD, see Fig. 2).

The analyses showed that (*Z*)-3-hexen-1-ol and 1-hexanol are major constituents of the birch-leaf volatiles from both *B. pubescens* (Fig. 2) and *B. pendula* (not shown, but about 27% and 19%, respectively, as much as *B. pubescens* from Lomma, Sweden). However, these compounds were not present in detectable amounts in bark odor (Fig. 2). (*Z*)-3-Hexen-1-ol and 1-hexanol were collected from *B. pubescens* leaves from Torsby, Sweden, at 1.2 and 2.64 mg/100 g fresh weight daily, which should be similar to the release rates from the leaves used in the field traps (Fig. 1). Thus these two compounds were released from the leaves in the field experiments at about 10% and 46% of the release rates for the neat compounds in the rotor traps, respectively. This means that the inhibition of both bark beetle species by birch-leaf volatiles is due in part to (*Z*)-3-hexen-1-ol and 1-hexanol. However, the reduced attraction to pheromone in both species by odors of birch bark probably cannot be explained by these two compounds as they were not detected in odor collections.

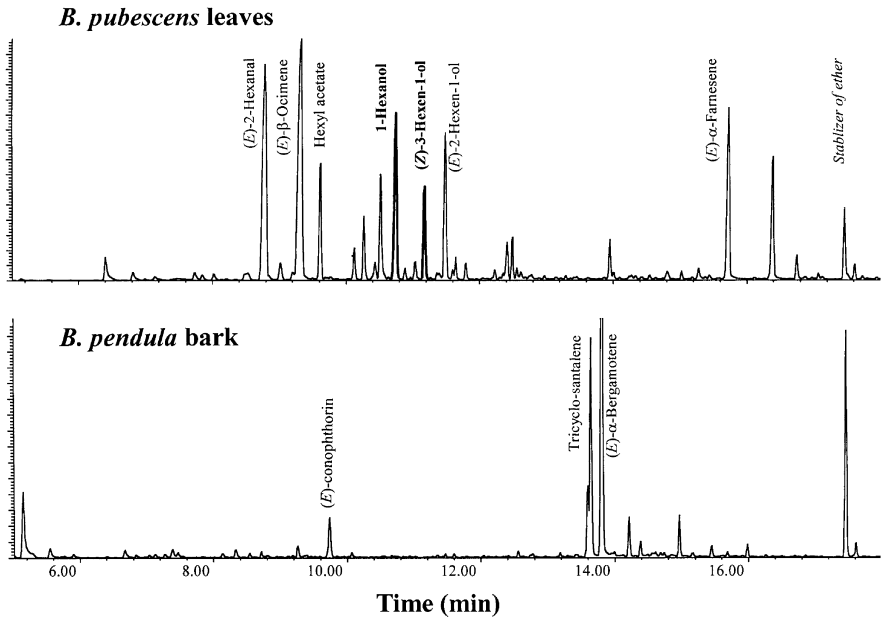


Fig. 2. Upper gas chromatogram (GC) of volatiles collected from air passed over leaves on terminal branches of *Betula pubescens* (cut 27 June 1997 and given water until ends of leafy shoots were placed in bags for volatile collection on 30 June 1997 at 27° C). Lower chromatogram of volatiles from air passed over bark chips of *B. pendula* (chips cut from tree on 27 June 1997 and held at 5° C until placed in bags for volatile collection 30 June 1997; leaves and bark from Torsby, Sweden). A fused-silica column, 0.25 mm × 25 m, coated with CP-wax 58 (PEG, 0.5 µm) CB was used for GC with helium as the carrier gas at a constant flow of 31 cm/s on a temperature program of 30° C for 3 min, a 10° C/min rise to 200°, and then constant for 10 min. Chemicals were identified by comparison of retention times and mass spectra to those of authentic compounds and computer data libraries (NBS75 K and KEM-EKOL)

Interestingly, we found that (*E*)-conophthorin, a spiroacetal, is released in significant amounts from bark of both birch species, but not from leaves (Fig. 2) or from Norway spruce bark. (*E*)-Conophthorin has previously been identified in European ash bark beetles (*Leperisinus varius*) and North American cone beetles (*Conophthorus resinosae* and *C. coniperda*) and inhibits attraction of these beetles to pheromones (Kohnle et al. 1992; Birgersson et al. 1995; Pierce et al. 1995). Conophthorin is also present in bark of North American *B. papyrifera* and several other deciduous trees and inhibits aggregation of two conifer bark beetles (*D. ponderosae* and *D. pseudotsugae*) of North America (J.H. Borden, personal communication). 1-Hexanol, found only in male *P. chalcographus* hindguts during feeding (Francke et al. 1977; Byers et al. 1990), may be a multifunctional semiochemical informing beetles of (a) likely competition at close-range on the host or (b) an unsuitable birch tree.

Nonhost trees (*B. pendula* and *Pinus sylvestris* of about 35 cm DBH) baited with synthetic pheromone of *I. typographus* (as in Fig. 1) attracted beetles, but few bored in, and then only a few millimeters, compared with hundreds of galleries in host spruce trees similarly baited (10–14 June 1997). Two smaller birch, *B. pendula* and *B. pubescens*, (14 cm DBH) were

also baited with *P. chalcographus* pheromone, and this caused numerous beetles to land on the trunk near the bait, but none was observed on several other birch of similar size standing 1.5–3 m away. Based on an average of $6.2 \pm 0.9$ ($\pm$95% CL, $n = 20$) beetles observed on the *B. pendula* trunk during the main flight period (12:00–17:00, 20–24° C) and on an average time before leaving of $121 \pm 57$ s ($\pm$95% CL, $n = 20$), assumed to be half the average landing time, at least 460 landed per day. However, no beetles were observed to bite into the bark, and not one attack was found in either species over the 4-day baiting periods. When 60 males of *P. chalcographus* and *I. typographus* were confined until death with *B. pendula* logs (12 cm diameter, 28 cm) in the laboratory, 37% and 23% bored in, respectively, but only in the rough bark areas, and then only a few millimeters into the phloem. No breeding galleries resulted after addition of an equal number of females 24 h later compared with Norway spruce logs, where nearly all beetles made egg galleries.

The avoidance of odors of birch bark and green-leaf volatiles by *I. typographus* and *P. chalcographus* before landing in the experiments could mean that these bark beetles avoid flying into areas with nonhost trees. However, this question cannot be answered until volatile concentrations in the forest some distance from birch trees are measured. In mixed birch and spruce stands, and at some distance, the mixing of odors would not seem to provide a useful signal. Nevertheless, after landing on a birch tree, the concentration of volatiles at the bark surface is probably sufficient to induce beetles to leave soon, as observed in the experiments with pheromone-baited birch trees. Beetles that can recognize the nonhost more rapidly would be favored in evolution since they would save minutes to hours of time that otherwise would be needed to bore into the bark and taste, during which they would be exposed to enemies. The fitness benefits of a quick decision concerning host suitability after landing is obvious since bark beetles exhaust their fat reserves after only a few hours of flight (Botterweg 1982; Forsse and Solbreck 1985).

In addition to the use of nonhost odors, bark beetles can discern concentrations of verbenone and ethanol to determine the unsuitability of bark habitat. Verbenone is produced by some bark beetles (*Dendroctonus* and *Tomicus*), but not by *Ips* or *Pityogenes*, and may also be of microbial origin in decaying hosts (Byers and Wood 1980; Byers et al. 1984, 1989, 1990; Byers 1995). Verbenone signifies to several species of bark beetle that the host is not suitable because of colonization by conspecifics or competing species in the genera *Dendroctonus*, *Tomicus*, *Ips* (including *I. typographus*), and *P. chalcographus* (Byers and Wood 1980; Bakke 1981; Byers et al. 1984, 1989; Schlyter et al. 1989; Byers 1992, 1993, 1995; Wilson et al. 1996; Borden et al. 1997). Ethanol also indicates microbial fermentation and unsuitable habitat to be avoided for species colonizing living trees (Moeck 1970; Kimmerer and Kozlowski 1982; Klimetzek et al. 1986; Schroeder and Lindelöw 1989; Byers 1992). It is becoming apparent that bark beetles have a more complex chemical vocabulary than previously believed to aid them in their selection of host trees and gallery sites.


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