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Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors

Abstract The bark beetle, *Pityogenes bidentatus* (Coleoptera: Scolytidae), searches in mixed conifer and deciduous forests of northern Europe for suitable branches of its host, Scotch pine (*Pinus sylvestris*). We tested whether odors from several diverse nonhost trees and plants common in the habitat (e.g., mountain ash, *Sorbus aucuparia*; oak, *Quercus robur*; alder buckthorn, *Frangula alnus*; blueberry, *Vaccinium myrtillus*; raspberry, *Rubus idaeus*; and grass, *Deschampsia flexuosa*) would reduce attraction of the bark beetle to traps releasing its aggregation pheromone components in the field. Volatiles from leaves or bark of each of these plants significantly reduced attraction of the beetles to their pheromone. Odors collected from these nonhosts and analyzed by GC/MS contained monoterpenes, sesquiterpenes, and "green-leaf" alcohols, several of which (e.g. 1-octene-3-ol and β -caryophyllene) reduced attraction to pheromone in the

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field and elicited electroantennographic responses. In the laboratory, reproduction by the beetle was marginal in nonhost Norway spruce, *Picea abies*, and was absent in the other nonhost trees. Olfactory avoidance of unsuitable nonhosts may have evolved due to advantages of avoiding mistakes during host selection.

Introduction

Several species of bark beetles (Coleoptera: Scolytidae) have been shown to avoid various "green-leaf" alcohols (hexanol isomers) and monoterpenes associated with nonhost trees (review in Zhang 2003). However, only a few studies have tested effects of odors from portions of nonhost trees on bark beetle responses, measured emissions of volatiles from these nonhosts, or determined the reproductive suitability of nonhosts in order to clarify the ecological significance of the avoidance phenomenon (Byers et al. 2000). In addition, no studies have tested a wide array of nonhosts in the habitat for repellent effects on a bark beetle. Bark beetles may encounter and probe both host and nonhost trees and plants frequently while flying for hundreds of meters in search of suitable host trees (Byers 1996). Thus, it would be advantageous for bark beetles to have evolved olfactory-mediated behaviors for the avoidance of a wide variety of nonhost plants in order to save time and energy during a search.

Pityogenes bidentatus (Herbst) is a small, 2-mm long bark beetle that is common in forests of northern Europe. The beetle aggregates in groups that colonize weakened or diseased branches of host Scotch pine, *Pinus sylvestris* L. Healthy Scotch pine, and nonhost conifers such as Norway spruce, *Picea abies* (L.), are not attacked by *P. bidentatus*, apparently due to their avoidance of monoterpene vapors from toxic tree resins (Byers et al. 2000). Birch, *Betula pendula* Roth, also is unsuitable for colonization (Byers et al. 2000), either because of chemical resistance or a lack of nutritional constituents. Odors from birch and several of its chemical components, including 1-hexanol, *Z*-3-hexen-1-ol, *E*-2-hexen-1-ol, and various monoterpenes, are avoided by *P. bidentatus* in the field (Byers et al. 2000).

In southern Sweden, some of the most common nonhost trees of *P. bidentatus* are birch (*B. pendula*, Betulaceae), mountain ash (*Sorbus aucuparia* L., Rosaceae), oak (*Quercus robur* L., Fagaceae), and alder buckthorn (*Frangula alnus* P. Mill., Rhamnaceae). In addition, there are common nonhost plants such as blueberry (*Vaccinium myrtillus* L., Ericaceae), raspberry (*Rubus idaeus* L., Rosaceae), and grass (*Deschampsia flexuosa* (L.) Trin., Poaceae) that *P. bidentatus* may encounter near storm-felled host trees and its branches.

Our objective was to determine whether *P. bidentatus*, while orienting to pheromone, avoids odors released from the common nonhost trees and plants.

Furthermore, we wanted to collect the volatile emissions for identification and quantification by GC/MS and observe behavioral and electroantennographic responses. We also wanted to determine whether the beetles were able to reproduce in the nonhost trees, since an inability to do so would provide evidence of a strong selection pressure in the evolution of the behavioral avoidance of nonhost volatiles.

Materials and methods

To investigate whether *P. bidentatus* has evolved strategies for avoiding nonhost plants during host finding, we tested the effects of volatiles from leaves or bark of trees and plants from the habitat on the attraction of *P. bidentatus* to its aggregation pheromone components, grandisol and *cis*-verbenol (Byers et al. 2000), in the field. The pheromone baits were placed inside four pairs of rotating traps. Each trap consisted of a plastic cylinder (18-cm diam. x 28-cm high) covered at the top and open at the bottom, suspended over a funnel (31-cm diam.) that collected beetles striking the cylinder (Fig. 1). Inside each cylinder was a fine screen cage, either empty (control) or containing bark or twigs with leaves from four nonhost trees of each species (mountain ash, oak, and alder buckthorn) or from each of five or more nonhost plant species (i.e., blueberry, raspberry, or grass) found in the habitat (Fig. 1). Each pair of traps was separated 6-m apart at 1.2-m height and revolved slowly at 2 rph, which minimized spatial variations in insect densities allowing Chi-square (χ^2) tests with one degree of freedom (Byers et al. 1998, 2000). The plant materials were each divided into two equal portions for use in one of the paired traps and for volatile collections.

Volatiles from the leaves/twigs or bark sections were collected for chemical analyses by enclosing them in polyacetate bags (Meny Toppits®, 35 x 43 cm) through which activated-carbon-filtered air was drawn at 300 ml/min. The effluent volatiles were adsorbed on 30 mg Porapak Q (50-80 mesh, Supelco) in a 3-mm ID Teflon tube for 1.5 h. Diethyl ether washings (300 μ l) 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 spectrometer (GC-MS). Columns, conditions, and quantification procedures were as described in Zhang et al. (1999). Three μ l of aeration samples of mountain ash leaves were injected splitless into an HP 6890 GC equipped with an HP-INNOWAX column (30 m x 0.25 mm x 0.25 μ m) and a 1:1 effluent splitter that allowed simultaneous flame ionization detection (FID) and electroantennographic detection (EAD) of volatile compounds (Zhang et al. 2000). EAD signals were analyzed on a PC equipped with an IDAC-card and software version 2.3 from Syntech (Hilversum, The Netherlands). We also tested male and female antennae to a synthetic mixture containing 10-nonhost compounds (100 ng/ μ l each) and two pheromone components (5 ng/ μ l each).

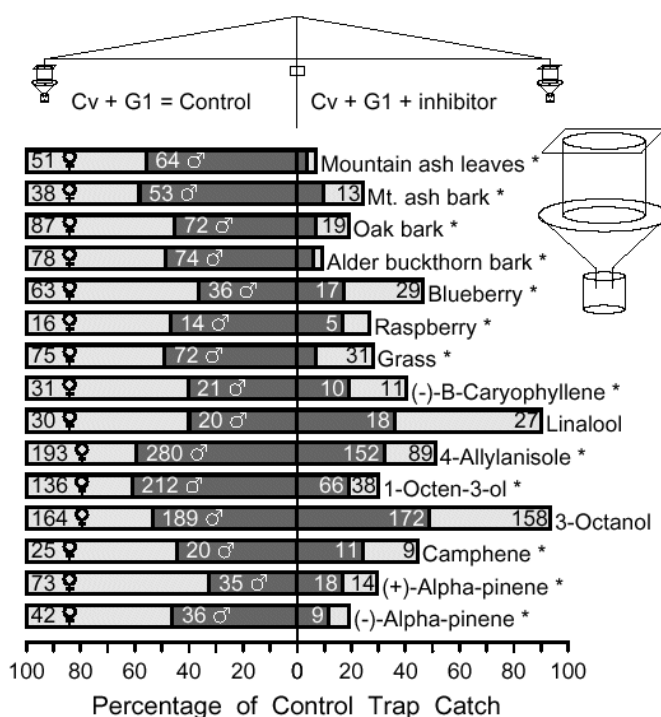


Fig. 1. Catch of *Pityogenes bidentatus* on traps releasing pheromone plus inhibitor volatiles compared to control traps with pheromone alone. The trap pairs were mechanically-rotated at 2 rph to minimize catch variation due to trap position. Test replicates were conducted for at least 1 hour, and after 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 with a Chi square goodness of fit test to an expected catch if there were no differences based on the average for both traps. Tests used about 100 g (fresh weight) of bark strips or leaves/twigs (9-12 May 2001), and the chemicals as indicated (27 April - 26 May 2001) near Sjöbo, Sweden. *P. bidentatus* pheromone components, cV [(S)-*cis*-verbenol, 96%, Borregaard] and G1 [racemic *cis*-configured grandisol, (1R*,2S*)-1-(2-hydroxyethyl)-1-methyl-2-(1-methylethenyl)cyclobutane, Frank Enterprises, Inc., Columbus, Ohio], were released from small tubes at 21 and 0.5-2 μ g/h, respectively. (-)-*trans*- β -Caryophyllene (>99%, Sigma), linalool (97%, Aldrich), 4-allylanisole (98%, Aldrich), 1-octen-3-ol (>99%, ACROS), and 3-octanol (98%, ACROS) were released at 20, 160, 270, 230, and 200 μ g/h, respectively. The "monoterpenes", (-)- α -pinene ($[\alpha]_D^{20} = -50^\circ$, >99.5%, Fluka), (+)- α -pinene ($[\alpha]_D^{20} = +57^\circ$, >99%, Fluka), and camphene ($[\alpha]_D^{23} = -6.6^\circ$, 99%, Aldrich) were released alone at about 1400, 1400, and 1075 μ g/h, respectively, from various combinations of tubes. Catches with asterisks were significantly lower than the pheromone controls in the same test at $P < 0.01$ (Chi-square goodness of fit).

To determine if bark of the nonhost trees was un-suitable for reproduction in *P. bidentatus*, groups of 20 males and 20 females, collected from the baited traps in the field, were released in a plastic box at room temperature containing two or three bolts (each 28-cm x 6- to 8-cm diam.) of either oak, birch (*B. pendula*), alder buckthorn, mountain ash, or host tree, Scotch pine (24 May 2001). The presence of dead beetles, boring dust, and new progeny was monitored weekly for two months.

Results

The odor from mountain ash (*S. aucuparia*) bark or leaves/twigs significantly reduced the response of *P.*

Table 1 Release rates of volatiles ($\mu\text{g/h}$) from plant materials (leaves and bark of nonhosts mountain ash, *Sorbus aucuparia*, grass, *Deschampsia flexuosa*, raspberry, *Rubus idaeus*, and alder buckthorn, *Frangula alnus*, used in the field test of *Pityogenes bidentatus* inhibition to aggregation pheromone (9-12 May 2001, Fig. 1). The volatiles were collected on Porapak Q and identified by GC-MSD^a.

Compound	Mountain ash: <i>S. aucuparia</i>		Grass: <i>D.</i> <i>flexuosa</i>	Raspbe rry: <i>R.</i> <i>idaeus</i>	Alder buckthorn : <i>F. alnus</i>
	Leaves	Bar k		Leaves	Bark
α -Pinene	0.15	0.1	0.3	0.05	
Camphene	0.08				
β -Pinene			0.06		
3-Carene			0.09		
α -Ocimene	0.06				
β -Ocimene	1.4				
(E)-4,8-Dimethyl- 1,3,7-nonatriene	0.6				
Pentyl acetate			0.07		
Z-2-Pentenyl acetate			0.2		
Hexyl acetate			1.3		
(Z)-3-Hexenyl acetate	0.2		42	0.2	
(E)-2-Hexenyl acetate			0.5		
6-Methyl-5- hepten-2-one			0.04		
(Z)-3-Hexenyl butanoate	0.04				
1-Hexanol		0.06	0.1		
(Z)-3-Hexen-1-ol			2.8		
1-Octen-3-ol			0.09		
Isopentyl propionate			0.1		
3-Ethyl-3-pentanol					0.07
Nonanal					0.05
Benzaldehyde	0.07	0.9			
Benzyl alcohol		0.09			

Linalool	0.7
β -Bourbonen	0.07
β -Caryophellene	0.2
α -Cubebene	0.1
β -Cubebene	1
α -Farnesene	0.5
Methyl salicylate	0.4

^aChemicals were identified by comparison of retention times and mass spectra to those of authentic compounds and computer data libraries (NBS75K and additions).

bidentatus to its aggregation pheromone (*cis*-verbenol and grandisol) compared to aggregation pheromone alone in the revolving trap pair ($\chi^2 > 42$, $df=1$, $P < 0.001$, Fig. 1). Similarly, bark volatiles from either oak (*Q. robur*) or alder buckthorn (*F. alnus*) dramatically reduced attraction to aggregation pheromone ($\chi^2 > 88$, $df=1$, $P < 0.001$, Fig. 1). Leaves of these two trees were not tested since they were not present in early May when the main flight of the beetle occurred. It was possible to observe orientation behavior of beetles in the late afternoon sun as they flew upwind for several meters converging toward the pheromone source. However, when the beetles approached to within about 0.5 to 1 m from the trap releasing tree volatiles, they were repelled and turned away. In contrast, beetles that approached the control baits flew upwind until they reached the source.

Analysis of the Porapak Q entrapped volatiles from the bark and leaf samples tested in the field revealed that mountain ash leaves and grass had the greatest quantities of volatiles, while lesser amounts of compounds were quantified from bark of mountain ash and alder buckthorn, and leaves of raspberry (Table 1). The amounts released by oak bark were too low for reliable identification, while blueberry odors were not collected. Monoterpenes were released from mountain ash (α -pinene, camphene, β -ocimene), grass (α - and β -pinene, 3-carene), and raspberry (α -pinene); sesquiterpenes (e.g. β -caryophellene and β -cubebene) from leaves of mountain ash, and green-leaf alcohols and esters (e.g. 1-hexanol, Z-3-hexenyl acetate, Z-3-hexen-1-ol) from mountain ash and grass (Table 1). Only a few volatiles were identified from raspberry and bark of alder buckthorn. Background air from the study area did not contain detectable amounts of the volatiles reported from the tree or plant samples.

Some of the monoterpenes quantified in odors from the plant materials in the field test, or emitted from trees in earlier work (Byers et al. 2000), were released in similar quantities as synthetics in the rotating pair of traps (Table 1, Fig. 1). The monoterpenes, camphene ($\chi^2 = 9.6$, $df=1$, $P = 0.002$) and both enantiomers of α -pinene ($\chi^2 > 41$,

$df=1$, $P<0.001$), reduced responses significantly (Fig. 1). (-)- β -Caryophyllene also reduced the response of *P. bidentatus* to pheromone ($\chi^2=13.2$, $df=1$, $P<0.001$), while linalool (from mountain ash) had apparently no significant effect at the release rate tested ($\chi^2=0.26$, $df=1$, $P=0.61$). 4-Allylanisole, found in several pine species, and reported to reduce responses of bark beetles in North America (Joseph et al. 2001) also reduced responses of *P. bidentatus* ($\chi^2=75$, $df=1$, $P<0.001$). 1-Octen-3-ol found in grass (Table 1), birch, and aspen (Zhang et al. 2000) reduced attraction of *P. bidentatus* ($\chi^2=132$, $df=1$, $P<0.001$, Fig. 1).

Gas chromatography combined with electroantennographic detection (GC-EAD) of a synthetic mixture showed that the strongest antennal responses were to the two pheromone components, followed by green leaf alcohols (1-hexanol, *Z*-3-hexen-1-ol, and *E*-2-hexen-1-ol), C_8 -alcohols and other common angiosperm nonhost volatiles (Fig. 2A). Most of the antennally-active nonhost volatiles were shown to be behaviorally inhibitory (Fig. 1; Byers et al. 2000). GC-EAD also showed that several volatiles from leaves of mountain ash, linalool, β -caryophyllene, β -cubebene, α -farnesene, and an unidentified sesquiterpene, elicited electrical signals in the antenna of *P. bidentatus* (Fig. 2B). A common deciduous tree volatile, *trans*-conophthorin, elicited a small EAD response (Fig. 2A) but was not active in the field (Byers et al. 2000). No differences in antennal responses between males and females were detected, nor were there significant differences in sex ratios of catch on baits with or without nonhost trees and shrubs (all $\chi^2<2$, $df=1$, $P>0.05$, Fig. 1).

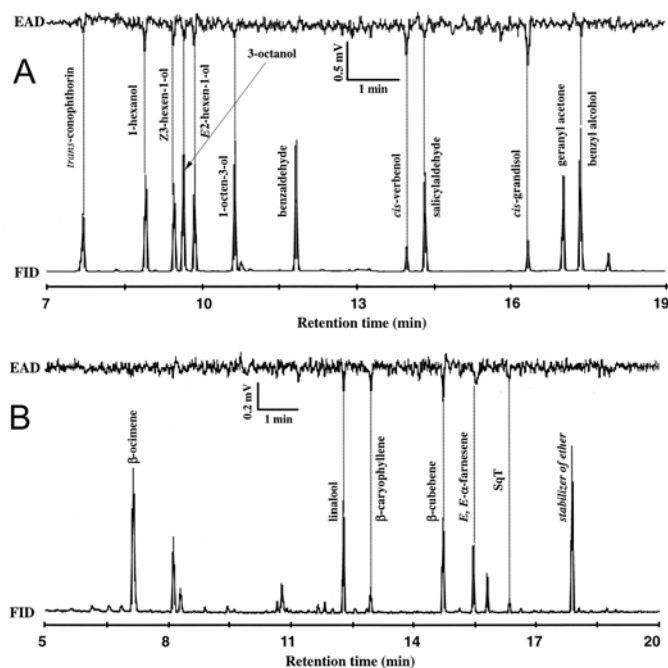


Fig. 2A, B. Electroantennographic detector (EAD) responses of *Pityogenes bidentatus* to elution of compounds from a gas chromatograph as measured by a flame ionization detector (FID).

In A, the largest responses were elicited by the *P. bidentatus* pheromone components *cis*-verbenol and *cis*-grandisol, the plant volatiles 1-hexanol (>98%, Aldrich; found in odor from birch, mountain ash, and grass), *E*- and *Z*-3-hexen-1-ol (>98%, Aldrich; from birch and grass) gave intermediate responses, while *trans*-conophthorin (85%, Pherotech; from birch), 3-octanol (98%, ACROS), 1-octen-3-ol (>99%, ACROS; from grass), salicylaldehyde (>99%, Fluka), and benzyl alcohol (>99%, Aldrich; from mountain ash) showed lower activity. In B, 1% of the extract of mountain ash leaves and twigs elicited intermediate EAD signals to linalool, β -caryophyllene (also from birch and Norway spruce), and β -cubebene (also from birch), while α -farnesene had lower activity.

The nonhost logs were not suitable for colonization by *P. bidentatus*. In alder buckthorn, no attacks were made, while in oak, birch, and mountain ash, less than 50% of the beetles bored into the phloem at the cut ends of the logs. However, no beetles penetrated the bark more than a few mm and all were dead after 20 days. In contrast, in Scotch pine, all males were successful in making attacks, as indicated by piles of boring dust spread over the bark surface, and together with the females produced 232 progeny. In another experiment, Norway spruce and pine logs infested similarly with *P. bidentatus* produced comparable numbers of progeny for the first generation. However, by the third generation, only the pine logs produced significant numbers of progeny (180 versus 10 in spruce).

Discussion

Several species of bark beetle may avoid nonhosts as a means of finding hosts more quickly while avoiding possible damage during a mistaken attack of nonhosts (Schroeder 1992; Borden et al. 1998; Byers et al. 1998, 2000; Zhang 2003). Our study demonstrates that *P. bidentatus* avoids volatiles from more nonhost trees (birch, mountain ash, Norway spruce, oak, and alder buckthorn) and plant species (blueberry, grass, and raspberry) than any other bark beetle studied (Fig. 1). None of the tree and plant species reported here have been shown previously to be avoided by bark beetles. Sensory modalities other than olfaction, such as vision and gustation, may aid the bark beetle in avoiding nonhost plants. Furthermore, the volatile amounts released from cut tissues in our tests were probably higher than would occur from intact plants that usually would be encountered. Therefore, it may be that beetles primarily discriminate nonhosts after landing, where concentrations of inhibitory volatiles at the plant surface are expected to be vastly higher than just a few cm further out. However, an aggregation pheromone normally would not compete with a repellency effect from nonhost volatiles; unfortunately, a repellency effect of nonhost

chemicals in the absence of attractants is not practical to observe.

Many of the same chemicals found to be inhibitory to *P. bidentatus* in our study are present in other nonhost trees in the habitat. For example, birch, *B. pubescens*, aspen, *P. tremula*, and elder, *Sambucus nigra*, release significant amounts of the green leaf alcohols (e.g. 1-hexanol, *E*-2- and *Z*-3-hexen-1-ol) and monoterpenes (e.g. α - and β -pinene, 3-carene) comparable to that released from *B. pendula* and mountain ash (Fig. 2; Byers et al. 1998, 2000; Zhang et al. 1999). Monoterpenes are present in a number of plants, especially the conifers, so volatiles from all of these species may reduce response of *P. bidentatus* to aggregation pheromone. In fact, volatiles from host bark or needles of Scotch pine were repellent to *P. bidentatus*, probably as a means to avoid healthy trees and select diseased branches that are unable to exude defensive resin containing monoterpenes (Byers et al. 2000).

Our study is the first to report a sesquiterpene being inhibitory to bark beetles. (-)-*trans*- β -Caryophyllene reduced the response of *P. bidentatus* to aggregation pheromone, and was shown to be released from mountain ash. This sesquiterpene is also released from aspen, Norway spruce, elder and both species of birch (Zhang et al. 1999; Byers et al. 1998, 2000). Therefore, β -caryophyllene, and perhaps other sesquiterpenes released from angiosperm nonhost trees (Table 1; Zhang et al. 1999; Byers et al. 2000) may aid *P. bidentatus*, and possibly other conifer bark beetles, in avoiding nonhosts. Aggressive bark beetles such as *I. typographus* that encounter large quantities of monoterpenes and sesquiterpenes during tree colonization appear indifferent to these volatiles in flight (Byers unpublished; Byers et al. 2000).

Avoidance of nonhost odors may have evolved in *P. bidentatus* because it cannot reproduce in these species. The laboratory experiment that forced *P. bidentatus* to attempt colonization in several tree species indicates that the insect will not attempt attack of nonhost alder buckthorn, and if beetles attacked nonhosts oak, birch, and mountain ash, reproduction would be unsuccessful. In the case of Norway spruce, a conifer nonhost, *P. bidentatus* was not induced to attack in the field by pheromone baiting (Byers et al. 2000), but in the laboratory the beetle did attack and reproduce approximately normally although only for the first generation. Attractive pheromone baits placed on birch could not induce a sibling spruce-infesting species, *P. chalcographus*, to attack (Byers et al. 1998). *P. bidentatus* has evolved behaviors to avoid not only

nonhosts but also ethanol, representing an unsuitable host undergoing fermentation, but also chalcogran, a pheromone component of a competing bark beetle, *P. quadridens* (Byers et al. 2000).

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