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Antennal responses of the western pine beetle, *Dendroctonus brevicomis* (Coleoptera: Curculionidae), to stem volatiles of its primary host, *Pinus ponderosa*, and nine sympatric nonhost angiosperms and conifers

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Summary. Stem volatile extracts from ten trees that are sympatric with the western pine beetle, *Dendroctonus brevicomis* LeConte (Coleoptera: Curculionidae) were assayed by gas chromatographic-electroantennographic detection analysis (GC-EAD). The extracts were from the primary host, ponderosa pine, *Pinus ponderosa* Dougl. ex Laws. (Pinaceae); two nonhost angiosperms, California black oak, *Quercus kelloggii* Newb. (Fagaceae), and quaking aspen, *Populus tremuloides* Michx. (Salicaceae); and seven nonhost conifers, white fir, *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr. (Pinaceae), incense cedar, *Calocedrus decurrens* (Torr.) Florin (Cupressaceae), Sierra lodgepole pine, *P. contorta murrayana* Grev. & Balf. (Pinaceae), Jeffrey pine, *P. jeffreyi* Grev. & Balf. (Pinaceae), sugar pine, *P. lambertiana* Dougl. (Pinaceae), Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae), and mountain hemlock, *Tsuga mertensiana* (Bong.) Carr. (Pinaceae). Sixty-four compounds were identified from the ten trees, 42 of which elicited antennal responses in *D. brevicomis*, usually in both sexes. In addition, several synthetic compounds, including a number of the antennally-active compounds from the extracted trees and some bark beetle pheromone components, elicited antennal responses in a manner similar to that observed with the extracts. Of the antennally-active compounds known to be present in trees sympatric with *D. brevicomis*, only geraniol was unique to its host. Four antennally-active compounds were found in the host and in other conifers; five compounds were found only in nonhost conifers; eight compounds were found in either or both of the nonhost angiosperms; eight compounds were found in either or both of the angiosperms and in nonhost conifers, but not in the host; and 19 were found in both the host and in angiosperms and/or nonhost conifers. Several bark beetle pheromone components were found in the stem volatile extracts. Conophthorin was identified from both nonhost angiosperms; *exo*-brevicomin was identified in *A. concolor*; verbenone was identified from a number of nonhost conifers; and chalcogran was identified from *P. tremuloides*. The number of nonhost volatile chemicals that *D. brevicomis* encounters and is capable of detecting, and the diversity of sources from which they emanate, highlight the complexity of the olfactory environment in which *D. brevicomis* forages. This provides a basis for further work related to chemically-mediated aspects of foraging in this insect and perhaps other coniferophagous bark beetles, and highlights the need to consider foraging context in the design and implementation of semiochemical-based management tactics for tree protection.

Key words. Insecta – Coleoptera – Curculionidae – Scolytinae – chemical ecology – dispersal – kairomone – optimal foraging – pheromone – synomone

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Introduction

After spending the entire immature portion of their life cycle under the bark of a host tree, newly emerged adult bark beetles (Coleoptera: Curculionidae) leave the protection of the brood host and search for a new host in which to reproduce (Rudinsky 1962). The diverse mixture of trees present in the foraging area of most bark beetles, combined with the relatively narrow host range of many of these insects (Wood 1982) and the high costs associated with mistakes (Atkins 1966), implies that they should be able to detect and respond to olfactory cues from potential hosts and nonhosts in their foraging area in order to successfully and efficiently locate new hosts. Since foraging adult bark beetles maintain very limited energy reserves (Atkins 1969) and are highly susceptible to predation (Stephen & Dahlsten 1976; Dahlsten 1982) and abiotic conditions (McMullen & Atkins 1962; Gries *et al.* 1989) during dispersal, there are clear advantages to discriminating among hosts and nonhosts from a distance.

The ability of coniferophagous bark beetles to detect and respond to volatiles known to emanate from host and nonhost trees has been well-researched for a number of species (Lanne *et al*. 1987; Byers 1995; Zhang & Schlyter 2004; Seybold *et al.* 2006). There is significant evidence that coniferophagous bark beetles are capable of responding in flight to complex mixtures of synthetic nonhost angiosperm volatiles (NAVs) (Dickens *et al.* 1992; Wilson *et al.* 1996; Borden *et al.* 1997; Byers *et al.* 1998; Deglow & Borden 1998a,b; Poland *et al.* 1998; Huber *et al.* 1999; 2000a,b; 2001; Zhang *et al.* 1999a,b; 2000; 2001; 2002; Poland & Haack 2000; Huber 2001; Huber & Borden 2001a,b; 2003; Jactel *et al.* 2001; Zhang 2001; 2003; Zhang & Schlyter 2003; Fettig *et al.* 2005). Although foraging bark beetles also land on and directly test hosts and nonhosts by taste or close-range olfaction (Elkinton & Wood 1980; Moeck *et al.* 1981), the ability to detect and avoid nonhosts without landing reduces the likelihood of negative interactions between foraging bark beetles and various generalist predators and parasitoids that may be associated with the surface of nonhost trees (Stephen & Dahlsten 1976; Dahlsten 1982). Exploitation of this flight behavior in these economically- and ecologically-important insects has recently been studied by using NAVs and other antiaggregants to reduce attack on single trees and in forest stands (Wilson *et al.* 1996; Borden *et al.* 1998; 2003; Huber & Borden 2001b; Jakuš *et al.* 2003; Zhang & Schlyter 2004; Fettig *et al*. 2007).

Although some NAVs appear to be antennally-active in a number of coniferophagous bark beetles (Huber *et al.* 2000b), there is enough variability in both antennal and behavioral responses (Dickens *et al.* 1992; Wilson *et al.* 1996; Borden *et al.* 1997; Byers *et al.* 1998; Deglow & Borden 1998a,b; Poland *et al.* 1998; Huber *et al.* 1999; 2000a,b; 2001; Zhang *et al.* 1999a,b; 2000; 2001; 2002; Schlyter *et al*. 2000; Poland & Haack 2000; Huber 2001; Zhang 2001; 2003; Huber & Borden 2001a,b; 2003; Jactal *et al.* 2001; Zhang & Schlyter 2003; Fettig *et al.* 2005) to justify the use of detailed coupled gas chromatographic-electroantennographic detection (GC-EAD) analyses to guide the implementation of efficient trapping bioassays in the field.

The western pine beetle, *Dendroctonus brevicomis* LeConte (Coleoptera: Curculionidae), is a major cause of mortality of ponderosa pine, *Pinus ponderosa* Dougl. ex Laws., in much of western North America, particularly in California (Furniss & Carolin 1977). Under certain conditions, this species can aggressively attack and kill apparently healthy trees of all ages and size classes (Miller & Keen 1960). In the case of *D. brevicomis*, all research into its responses to NAVs has relied upon analogous antennal or behavioral responses by other coniferophagous bark beetles (Fettig *et al.* 2005) or upon knowledge of angiosperm volatiles in general (Poland *et al.* 1998). Previously, we have shown that some complex groups of NAVs, when combined with the antiaggregation pheromone verbenone, disrupt the response of *D. brevicomis* to attractant-baited traps to levels significantly below that of verbenone alone (Fettig *et al.* 2005), and protect individual *P. ponderosa* from attack by *D. brevicomis* (Fettig *et al*. 2007). It is possible, however, that *D. brevicomis* antennae do not detect some of the NAV compounds in the complex mixtures that were tested, and that other antennally-active compounds have not yet been tested in the field against this insect. In addition, it is possible that some nonhost conifer volatiles (NCVs) may also be behaviorally-active in this insect. In order to determine accurately which compounds might play a role in this insect's foraging behavior, we explored *D. brevicomis* antennal responses to the stem volatiles of its primary host, to the stem volatiles of seven nonhost conifers and two nonhost angiosperms, and to 23 synthetic plant volatiles and bark beetle pheromone components. The results of this study provide a comprehensive list of plant-derived compounds that may be further tested for their behavioral effects on this important forest pest (Miller & Keen 1960; Furniss & Carolin 1977).

Materials and Methods

Collection and handling of beetles and host/nonhost material

Western pine beetle, *D. brevicomis*, and its primary host, *P. ponderosa*, were collected from one site in California (Table 1). Five nonhost trees were also collected from this site, whereas three other nonhost trees were collected from a second site in California (Table 1). The final nonhost, *T. mertensiana*, was collected from Alaska (Table 1) for other purposes, but was included in the study because although its high elevation distribution is largely allopatric with *D. brevicomis*, there are potential zones of sympatry in California, Oregon, Washington, and British Columbia (Burns & Honkala 1990).

Larval, pupal, and adult *D. brevicomis* in outer bark and phloem were collected from standing *P. ponderosa* by falling trees and transporting ~1 m stem sections to the Chemical Ecology and Management of Forest Insects Laboratory in Davis, California where they were placed into wooden emergence cages (Browne 1972). Insects emerged into jars containing moist paper towels at 4°C and were immediately sorted by sex based on the presence of the female mycangium and protuberances on the male frons (Wood 1982). Beetles were shipped regularly in September through November 2005 by overnight mail on ice to the Southern Research Station in Pineville, Louisiana for GC-EAD analyses.

The stems of host and nonhost trees for collection of volatiles were harvested and sectioned into 2 m lengths for transportation to the laboratory in Davis, where they were further sectioned into 3-4 pieces, each 27–34 cm long and 7–12 cm diameter. *Tsuga mertensiana* was shipped by overnight mail from Alaska in 60 cm lengths, which were also sectioned as described above.

Volatile entrapment and preparation of extracts for GC-EAD analysis

The cut logs were placed in groups of three to five into 19 l Sylon CT-treated glass carboys (Sylon CT, Sigma-Aldrich, St. Louis, MO). Air (4 l/min) was drawn into the bottom of each sealed carboy for 168 hr through activated charcoal and exited through a glass column [(2.8 cm ID/3 cm OD × 31 cm length, ground glass 24/40 joint) with constricted, open ends (0.3 cm ID/0.5 cm OD)] containing 15 g Porapak Q (50/80 mesh size; Sigma-Aldrich) (Seybold *et al.* 1995). All tubing and column junctions were sealed with Teflon® tape and Parafilm M® (Pechiney Plastic Packaging, Inc., Neenah, WI).

After 168 hr, the columns were immediately sealed with Duraseal and Parafilm M®, and then stored at –80°C prior to extraction. Each Porapak-Q sample was extracted by transferring the Porapak into a larger glass column and washing it with approx. 350 ml of pentane (Product No. 6145-4, Mallinckrodt Specialty Chemicals Co., Paris, KY), which was collected into a 500 ml concentration flask with a 10 ml graduated receptacle. An internal standard (80.8 µg of 4-decanone in 1 ml pentane) (99% chemical purity; Sigma-Aldrich) and then boiling chips were added to each

Table 1. Collection data for *Dendroctonus brevicomis* and for logs from host and non-host trees harvested in California and Alaska, July and August, 2005.

Species	Locality	Date collected
Western pine beetle, *Dendroctonus brevicomis* LeConte (Curculionidae)	CA: Shasta County, Shasta-Trinity National Forest, McCloud Flats, near Pilgrim Creek Road, 41.28 °N, 122.09 °W, 1049 m elevation	12 July 2005
Ponderosa pine, *Pinus ponderosa* Dougl. ex Laws. (Pinaceae)	CA: Shasta County, Shasta-Trinity National Forest, McCloud Flats, near Pilgrim Creek Road, 41.36 °N, 122.96 °W, 1207 m elevation	2 August 2005[a]
White fir, *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr. (Pinaceae)	CA: Shasta County, Shasta-Trinity National Forest, McCloud Flats, near Pilgrim Creek Road, 41.31 °N, 122.04 °W, 1122 m elevation	2 August 2005[b]
Incense cedar, *Calocedrus decurrens* (Torr.) Florin (Cupressaceae)	CA: Shasta County, Shasta-Trinity National Forest, McCloud Flats, near Pilgrim Creek Road, 41.31 °N, 122.04 °W, 1122 m elevation	2 August 2005[b]
Sierra lodgepole pine, *Pinus contorta murrayana* Grev. & Balf.(Pinaceae)	CA: Lassen County, Lassen National Forest, near Highway 44, 40.50 °N, 121.00 °W, 1700 m elevation	2 August 2005[c]
Jeffrey pine, *Pinus jeffreyi* Grev. & Balf. (Pinaceae)	CA: Lassen County, Lassen National Forest, near Highway 44, 40.50 °N, 121.00 °W, 1700 m elevation	2 August 2005[c]
Sugar pine, *Pinus lambertiana* Dougl. (Pinaceae)	CA: Shasta County, Shasta-Trinity National Forest, near Highway 89, 41.25 °N, 122.09 °W, 1036 m elevation	2 August 2005[c]
Quaking aspen, *Populus tremuloides* Michx. (Salicaceae)	CA: Lassen County, Lassen National Forest, near Pitville Road, 40.63 °N, 121.13 °W, 1715 m elevation	2 August 2005[d]
Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae)	CA: Shasta County, Shasta-Trinity National Forest, McCloud Flats, near Pilgrim Creek Road, 41.31 °N, 122.04 °W, 1122 m elevation	2 August 2005[b]
California black oak, *Quercus kelloggii* Newb. (Fagaceae)	CA: Shasta County, Shasta-Trinity National Forest, McCloud Flats, near Pilgrim Creek Road, 41.31 °N, 122.04 °W, 1122 m elevation	2 August 2005[d]
Mountain hemlock, *Tsuga mertensiana* (Bong.) Carr. (Pinaceae)	AK: Kenai Peninsula Borough, near Silvertip Creek, 60.73 °N, 149.35 °W, 250 m elevation	30 August 2005[e]

[a]Volatiles were collected 8-15 August 2005
[b]Volatiles were collected 3-10 August 2005
[c]Volatiles were collected 12-19 August 2005
[d]Volatiles were collected 2-9 August 2005
[e]Volatiles were collected 7-14 September 2005

sample. The extract was concentrated to approx. 4 ml by Kuderna-Danish evaporative concentration (Kontes, Vineland, NJ) in a 50°C water bath. The flask was then rinsed and added to the concentrate. The combined solution (total volume approx. 8 ml) was transferred to a sealed vial and stored at –80°C. Approximately 1 ml of each sample was sent to the Pineville laboratory for GC-EAD analyses.

GC-EAD analysis

Using GC-EAD, *D. brevicomis* antennal responses were recorded to extracts from the host, *P. ponderosa*, and from all nonhosts (Table 1); to synthetic mixtures of host volatiles and nonhost volatiles (HVs and NHVs, respectively); and to several bark beetle pheromone components (Table 2). Techniques used were similar to those described in Asaro *et al.* (2004). Briefly, the tip of a glass pipette Ag/AgCl reference electrode, containing Beadle-Ephrusi Ringer solution (0.5% polyvinylpyrrolidone), was inserted into the base of a severed beetle head. One side of the club of one antenna was positioned flat against the tip of a similar recording electrode. Each beetle was presented with only one extract or synthetic mixture.

Each extract of stem volatiles was concentrated 10× (1 µl to 0.1 ml) under a stream of nitrogen prior to GC-EAD analysis. For tests of extracts of *Q. kelloggii*, *P. tremuloides*, and *T. mertensiana*, a 1 µl aliquot was injected splitless into an HP 5890 gas chromatograph (GC) containing an HP-INNOWax column (60 m × 0.25 mm ID × 0.25 µm film thickness; Agilent Technologies, Wilmington, DE), with injector temperature set at 200°C; all other extracts were injected split (10:1). The carrier gas was helium at a linear flow rate of 29 cm/s. The column temperature was held at

Table 2. Synthetic compounds tested on male and female *Dendroctonus brevicomis* antennae.

Compound	Source[a]	Purity (%)
4-allylanisole	Sigma-Aldrich Corp.	98
benzaldehyde	Sigma-Aldrich Corp.	>99
benzyl alcohol	Sigma-Aldrich Corp.	>99
exo-brevicomin (racemic)	Pherotech International Inc.	97
(E)-conophthorin (racemic)	Pherotech International Inc.	87
decanal	Sigma-Aldrich Corp.	99
frontalin (racemic)	Pherotech International Inc.	99
guaiacol	Sigma-Aldrich Corp.	98
heptanal	Sigma-Aldrich Corp.	95
hexanal	Sigma-Aldrich Corp.	98
1-hexanol	Sigma-Aldrich Corp.	98
(*E*)-2-hexenal	Sigma-Aldrich Corp.	98
(*E*)-2-hexen-1-ol	Sigma-Aldrich Corp.	96
(*Z*)-2-hexen-1-ol	Sigma-Aldrich Corp.	95
(*Z*)-3-hexen-1-ol	Sigma-Aldrich Corp.	98
ipsdienol (racemic)	Borregaard	>95
methyl salicylate	Sigma-Aldrich Corp.	>99
myrcene	Sigma-Aldrich Corp.	90
nonanal	Sigma-Aldrich Corp.	95
α-pinene (racemic)	Sigma-Aldrich Corp.	95
salicylaldehyde	Sigma-Aldrich Corp.	98
(-)-verbenone	Pherotech International Inc.	95

[a]Sigma-Aldrich Corp., 3050 Spruce St., St. Louis, MO 63103, USA; Pherotech International Inc., 7572 Progress Way, Delta, British Columbia, V4G 1E9, Canada; Borregaard, P.O. Box 162, NO-1701, Sarpsborg, Norway

35°C for 1 min, increased at 16°C/min to 80°C, and then increased at 8°C/min to 230°C for 8 min.

For analysis of synthetic compounds, a 1 µ1 aliquot of each mixture was injected split (20:1) into the GC, with injector temperature set at 200°C. The remaining instrument conditions and column type were as described above. For the mixture containing α-pinene, myrcene, *exo*-brevicomin, frontalin, and ipsdienol, the column temperature was held at 40°C for 1 min, increased at 16°C/min to 80°C, and then increased at 7°C/min to 230°C for 5 min. For all other mixtures the column temperature was held at 40°C for 1 min, increased at 16°C/min to 80°C, increased at 7°C/min to 100°C for 5 min, and then further increased at 7°C/min to 230°C for 5 min.

After passing through the column, the extract and synthetic sample effluents were further split (1:1) for simultaneous flame ionization (FID) and electroantennographic (EAD) detection. The temperature of the FID was 240°C. For EAD, the effluent was introduced into a humidified air stream flowing over the antennal preparation at a rate of 400 ml/min. Each synthetic chemical tested was injected at a concentration of 4 µg/µl, resulting in 0.1 µg delivered to each antenna. Extracts were tested on two to four male and female *D. brevicomis* antennae, with the exception of the extract of *T. mertensiana*, which was tested only on females, because they are the pioneer sex (Table 3). Each synthetic mixture was tested on four male and female *D. brevicomis* antennae.

EAD traces of antennal responses to extracts were summed by sex and tree species prior to analysis in order to enhance smaller responses that would be difficult to distinguish from the background noise within a single run. Compounds that elicited detectable responses were identified on an Agilent 6890-5973 coupled gas chromatograph-mass spectrometer (GC-MS) employing identical column, temperature program, and carrier gas conditions to those used for GC-EAD. In all cases, identifications were verified using retention time matching with known synthetic standards on the GC-MS. The responses to each synthetic plant volatile and pheromone were standardized as a percentage of the response to an internal standard, *endo*-brevicomin (4 µg/µl). *endo*-Brevicomin was chosen as an internal standard because it elicited consistent, strong antennal responses in *D. brevicomis* antennae and it did not co-elute with any other synthetic compounds used in the GC-EAD analyses. Other potential standards failed to meet both of these criteria. Antennal responses to a particular synthetic compound were identified as statistically significant for one sex if at least two of the four responses were greater than the 90th percentile of the background noise (measured from 50 noise peaks occurring between retention times of known compounds; binomial probabilities test, $\alpha = 0.05$).

Results

Sixty-four compounds were identified from the ten tree species in the survey (Table 3). Many of these compounds elicited antennal activity (e.g., female *D. brevicomis* antennae responded to numerous components of stem volatile extracts of *Q. kelloggii*, *A. concolor*, and *P. ponderosa*, Fig. 1). One compound (naphthalene) elicited antennal responses only from male *D. brevicomis*. Four compounds (camphene, β-cubebene, geraniol, and methyl salicylate) elicited antennal responses from females only. An additional 37 compounds elicited antennal responses from both males and females; the remaining 22 compounds did not elicit antennal responses from either sex (Table 3). The concentration of individual compounds often varied widely among extracts of the ten tree species and for some species they may have been present in concentrations below the threshold for antennal detection. In addition, some compounds that elicited antennal responses were not identified due to insufficient quantities in the samples or due to co-elution with other compounds.

Extracts of *C. decurrens*, *P. contorta murrayana*, and *T. mertensiana* each contained the most antennally-active compounds (27), followed by *P. jeffreyi*, *P. ponderosa*, *P. lambertiana*, and *Pseudotsuga menziesii* (24); *A. concolor*

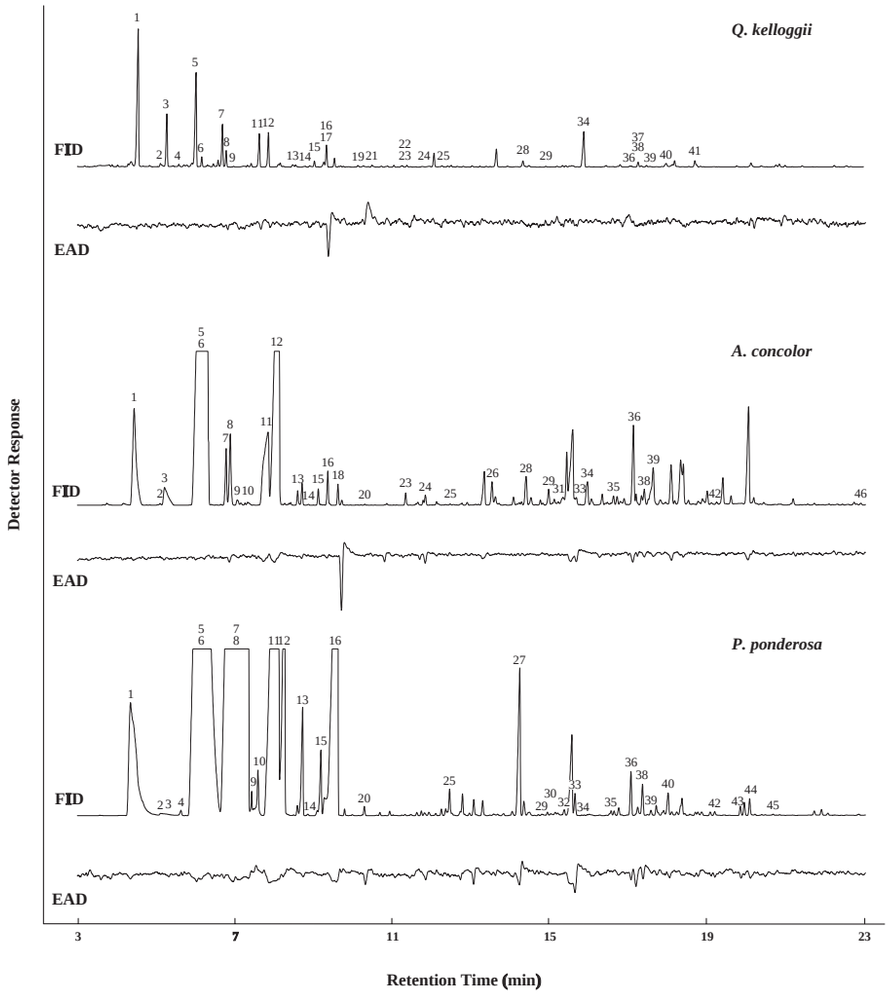

Fig. 1 Representative flame ionization detector (FID) and electroantennographic detector (EAD) traces of female *Dendroctonus brevicomis* antennal responses to compounds from stem volatile extracts in its primary host (*Pinus ponderosa*) and two nonhosts (*Quercus kelloggii*, *Abies concolor*). Each EAD trace represents the sum of three (*Q. kelloggii*) or four (*A. concolor, P. ponderosa*) individual runs. The following compounds were identified and verified: α-pinene (1), β-fenchene (2), camphene (3), undecane (4), α-pinene (5), sabinene (6), 3-carene (7), myrcene (8), α-phellandrene (9), α-terpinene (10), limonene (11), β-phellandrene (12), γ-terpinene (13), styrene (14), *p*-cymene (15), terpinolene (16), conophthorin (17), *exo*-brevicomin (18), 1-hexanol (19), 6-methyl-5-hepten-2-one (20), anisole (21), tetradecane (22), nonanal (23), fenchone (24), α-*p*-dimethylstyrene (25), decanal (26), linalool (27), camphor (28), isopinocamphone (29), fenchyl alcohol (30), bornyl acetate (31), pinocarvone (32), terpinen-4-ol (33), caryophyllene (34), myrtenal (35), 4-allylanisole (36), α-humulene (37), α-terpineol (38), borneol (39), β-cubebene (40), naphthalene (41), myrtenol (42), geraniol (43), *p*-cymene-8-ol (44), benzyl alcohol (45), and methyleugenol (46).

(23); and *Q. kelloggii* and *Populus tremuloides* (22). The monoterpenes camphene, 3-carene, *p*-cymene, limonene, myrcene, β-phellandrene, α-pinene, and β-pinene, were present in identifiable quantities in extracts of all ten tree species, i.e., including the two angiosperms. Antennally-active compounds identified uniquely from individual tree species included *exo*-brevicomin (*A. concolor*), chalcogran, (*Z*)-3-hexen-1-ol, salicylaldehyde, guaiacol, and phenylethyl alcohol (*P. tremuloides*); *trans*-pinocarveol (*P. lambertiana*); and geraniol (*P. ponderosa*) (Table 3).

The extracts of the two angiosperm tree species, *Q. kelloggii* and *P. tremuloides*, contained 11 compounds not found in any of the eight conifer extracts. Of these, chalcogran, conophthorin, guaiacol, (*Z*)-3-hexen-1-ol, phenylethyl alcohol, and salicylaldehyde, were antennally-active in both sexes of *D. brevicomis*. Seventeen compounds were identified among the conifer extracts that were not present in the angiosperm extracts, including several that elicited antennal responses. These active compounds included *exo*-brevicomin, fenchyl alcohol, bornyl acetate, terpinen-4-ol, *trans* pinocarveol, *trans*-verbenol, verbenone, myrtenol, geraniol, and *p*-cymene-8-ol (Table 3).

Geraniol was identified only in the extract of the primary host *P. ponderosa*, and it elicited a sex-specific response from female *D. brevicomis* antennae. Five antennally-active compounds were present in extracts of at least four nonhost conifer species, but not *P. ponderosa*: camphor, fenchone, 1-hexanol, nonanal, and verbenone. Of this group, all but verbenone were also found in extracts of one or both of the nonhost angiosperms.

Table 3. Summary of antennal responses of male and female *Dendroctonus brevicomis* to compounds identified from stem volatile extracts of its primary host, *Pinus ponderosa*, two nonhost angiosperm species (*Quercus kelloggii* and *Populus tremuloides*), and seven nonhost conifer species (*Pseudotsuga menziesii, Abies concolor, Calocedrus decurrens, P. lambertiana, P. contorta, P. jeffreyi*, and *Tsuga mertensiana*). All sample material, except *T. mertensiana*, collected in California, USA. All compounds were verified with synthetic standards.

		Tree species[b]									
Compound	**Antennal Responses[a]**	***P. ponderosa* (M-3; F-4)**	***Q. kelloggii* (M-3; F-3)[c]**	***P. tremuloides* (M-4; F-2)**	***P. menziesii* (M-3; F-2)**	***A. concolor* (M-4; F-4)**	***C. decurrens* (M-4; F-4)**	***P. lambertiana* (M-4; F-4)**	***P. contorta* (M-4; F-4)**	***P. jeffreyi* (M-4; F-4)**	***T. mertensiana* (M-0; F-4)**
4-allylanisole	M,F	+	+			+	+	+	+	+	+
anisole			+	+							
benzaldehyde	M,F			+					+	+	+
benzyl alcohol	M,F	+		+					+	+	+
borneol	M,F	+	+		+	+	+	+	+	+	+
bornyl acetate	M,F				+	+	+				
exo-brevicomin	M,F					+					
camphene	F	+	+	+	+	+	+	+	+	+	+
camphor	M,F		+			+	+	+	+		+
3-carene	M,F	+	+	+	+	+	+	+	+	+	+
caryophyllene		+	+	+	+	+	+	+			+
chalcogran	M,F			+							
conophthorin	M,F		+	+							
β-cubebene	F	+	+				+				+
p-cymene	M,F	+	+	+	+	+	+	+	+	+	+
p-cymene-8-ol	M,F	+					+	+	+		+
decanal						+				+	
α-*p*-dimethylstyrene	M,F	+	+		+	+	+	+	+	+	
α-fenchene		+	+	+	+	+	+	+	+	+	+
fenchone	M,F		+		+	+	+	+	+		+
fenchyl alcohol	M,F	+			+		+	+	+	+	
geraniol	F	+									
guaiacol	M,F			+							
heptanal				+							
hexadecane				+							+
hexanal				+						+	+
1-hexanol	M,F		+	+				+	+	+	+
(*E*)-2-hexenal				+							
(*E*)-2-hexen-1-ol				+							

Table 3. (Continued)

Compound	Antennal Responses[a]	Tree species[b]									
		P. ponderosa (M-3; F-4)	*Q. kelloggii* (M-3; F-3)[c]	*P. tremuloides* (M-4; F-2)	*P. menziesii* (M-3; F-2)	*A. concolor* (M-4; F-4)	*C. decurrens* (M-4; F-4)	*P. lambertiana* (M-4; F-4)	*P. contorta* (M-4; F-4)	*P. jeffreyi* (M-4; F-4)	*T. mertensiana* (M-0; F-4)
(*Z*)-3-hexen-1-ol	M,F			+							
α-humulene			+				+	+			+
isoborneol					+		+	+			
isopinocamphone	M,F	+	+		+	+	+	+	+	+	+
limonene	M,F	+	+	+	+	+	+	+	+	+	+
linalool	M,F	+		+	+					+	
methyleugenol						+			+	+	
6-methyl-5-hepten-2-one	M,F	+		+	+	+	+		+		+
methyl salicylate	F			+					+	+	+
myrcene	M,F	+	+	+	+	+	+	+	+	+	+
myrtenal		+			+	+	+	+	+		
myrtenol	M,F	+			+	+	+	+			
naphthalene	M		+	+			+		+	+	
nonanal	M,F		+	+	+	+			+	+	+
1-octanol										+	
(*E*)-2-octenal				+							
α-phellandrene		+	+		+	+	+	+	+		+
β-phellandrene	M,F	+	+	+	+	+	+	+	+	+	+
phenylethyl alcohol	M,F			+							
α-pinene	M,F	+	+	+	+	+	+	+	+	+	+
β-pinene	M,F	+	+	+	+	+	+	+	+	+	+
trans-pinocarveol	M,F							+			
pinocarvone		+			+		+	+	+		
sabinene		+	+		+	+	+	+	+	+	+
salicylaldehyde	M,F			+							
styrene		+	+	+	+	+		+	+	+	+
α-terpinene		+			+	+	+	+	+		+
γ-terpinene	M,F	+	+		+	+	+	+	+	+	+
terpinen-4-ol	M,F	+			+	+	+	+	+		+
α-terpineol	M,F	+	+		+	+	+	+	+	+	+
terpinolene	M,F	+	+		+	+	+	+	+	+	+
tetradecane			+	+	+				+		+
undecane		+	+	+	+			+		+	
trans-verbenol	M,F				+		+				
verbenone	M,F				+		+	+		+	+

[a] Positive antennal responses by male (M) and/or female (F) *D. brevicomis* elicited from at least one of the extracts containing a particular compound. Blanks indicate no measurable antennal response to a compound in any of the bark extract tests. Individual antennally-active compounds may have elicited responses in tests of one, some, or all of the bark extracts in which they were present.

[b] "+" = compound was present in bark extract of the particular tree species as identified by GC-MS, regardless of whether or not it elicited an antennal response.

[c] Number of male (M) and female (F) beetles tested for each tree species.

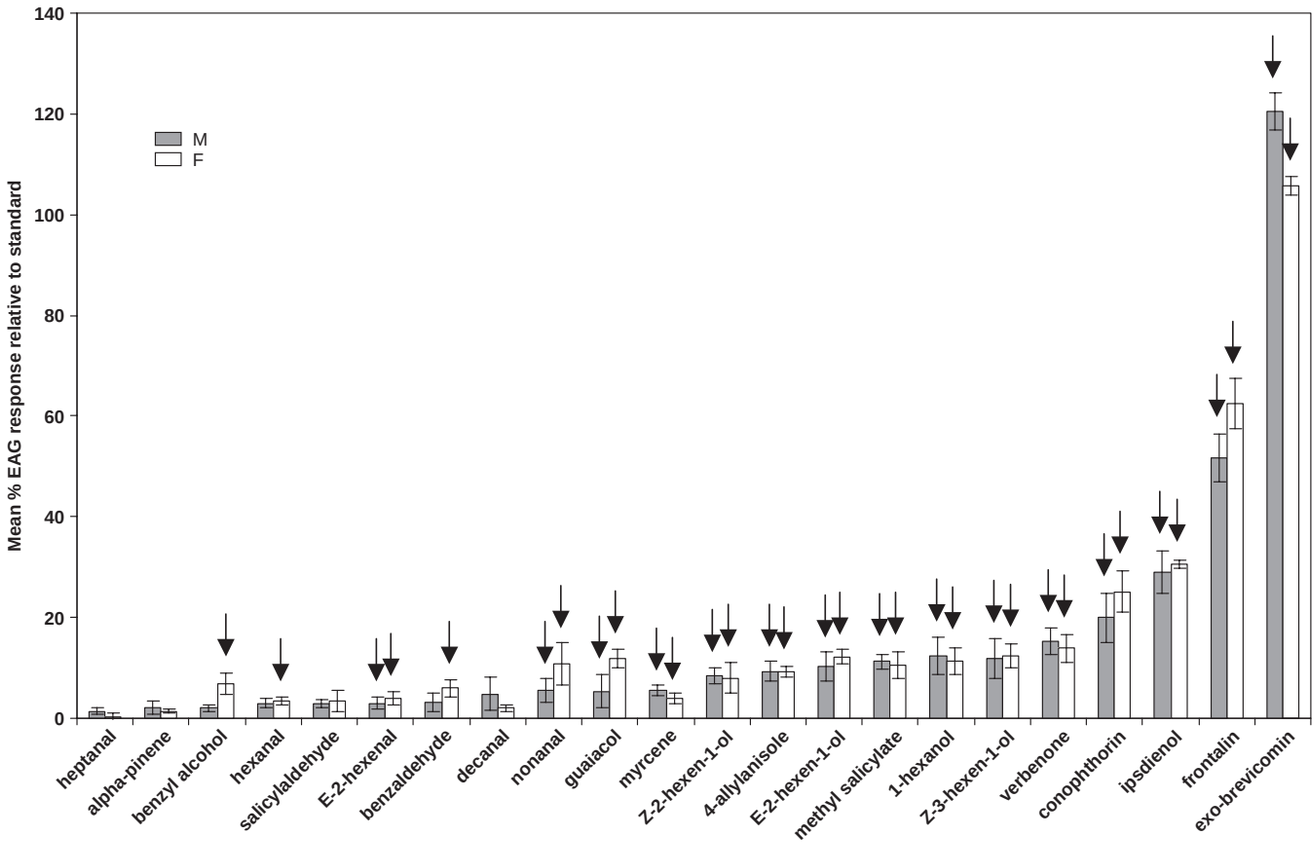

Fig. 2 Mean % EAGs (±SE) relative to the internal standard, *endo*-brevicomin (4 μg/μl), of male and female *Dendroctonus brevicomis* exposed to 0.1 μg of host and nonhost volatiles, and bark beetle-associated compounds (n = 4). Error bars depict standard errors of the means. Arrows above means indicate statistically significant responses (binomial probabilities test, $\alpha = 0.05$).

Eighteen of 22 synthetic compounds tested (Table 2) elicited statistically significant responses from antennae of *D. brevicomis* (Fig. 2). Only female antennae responded significantly to three of those compounds (benzyl alcohol, hexanal, and benzaldehyde). Both male and female antennae responded significantly to the remaining 15 compounds. The four compounds that did not elicit antennal responses were decanal, heptanal, α-pinene, and salicylaldehyde. The largest antennal responses were to known *D. brevicomis* pheromone components (*exo*-brevicomin, frontalin, ipsdienol, and verbenone) (Byers & Wood 1980; Byers 1982; 1983; Byers *et al.* 1984; Tilden & Bedard 1988; Bertram & Payne 1994) and to conophthorin, a known pheromone component of other bark beetles (Kohnle *et al.* 1992; Birgersson *et al.* 1995; Pierce *et al.* 1995; Dallara *et al.* 2000). Conophthorin and verbenone, which, in addition to being scolytid pheromone components are also known tree volatiles (Table 3; Huber *et al.* 1999), elicited the largest response among the synthetic NHVs tested. However, in general, responses to NHVs were smaller than to bark beetle pheromone components.

Twenty-one of the 22 synthetic compounds that were tested against *D. brevicomis* antennae were either also identified from extracts of at least one of the ten tree species (Table 3) or are known scolytid pheromones (Skillen *et al.* 1997). The remaining synthetic compound is the green leaf volatile (*Z*)-2-hexen-1-ol (Huber *et al.* 2000b), which elicited antennal responses (in both males and females) in *D. brevicomis*. When antennal responses to synthetic and naturally occurring compounds were compared, the presence or absence of response by both sexes was consistent for both classes of compounds if the responses were greater than or equal to that of nonanal. The exceptions were (*E*)-2-hexen-1-ol and methyl salicylate. Synthetic (*E*)-2-hexen-1-ol and methyl salicylate stimulated antennal responses in both male and female antennae, whereas in the natural extracts no responses to (*E*)-2-hexen-1-ol and only responses from females to methyl salicylate were observed, possibly due to dose effects. The responses to eight of the synthetic compounds were not identical as responses to the same compounds in natural extracts. Salicylaldehyde and α-pinene elicited no detectable antennal responses in synthetic form, but elicited responses from both sexes in the tree extracts. Benzyl alcohol and benzaldehyde elicited responses from females only in synthetic form, but from both sexes in the tree extracts. Antennal responses to (*E*)-2-hexenal (both males and females) and hexanal (only females) were observed with synthetic compounds, whereas in tree extracts no antennal responses to these compounds were detected, also possibly due to dose effects (Table 3, Fig. 2).

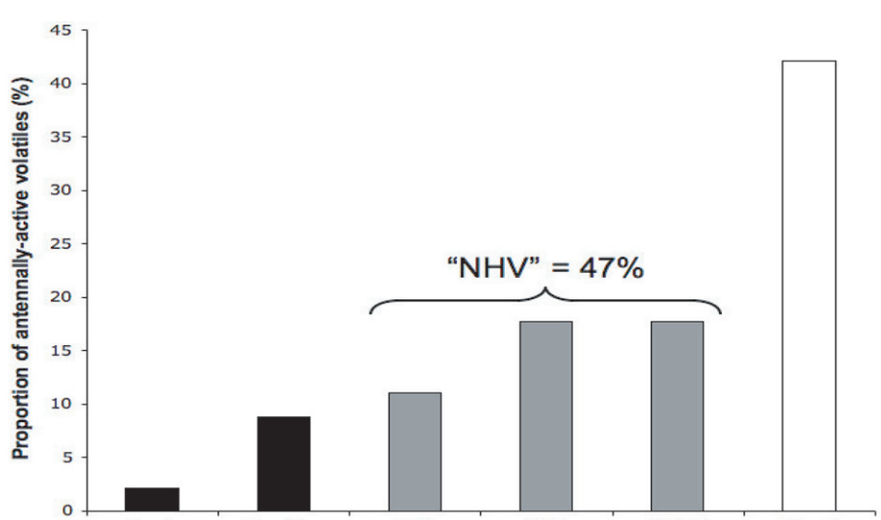

Fig. 3 Proportions of compounds (out of 47) that elicited EAD activity in male and/or female *Dendroctonus brevicomis* antennae. Source of compounds was from either or both natural extracts and synthetic chemicals, which were classified in six general categories. Host = found only in *Pinus ponderosa*; Conifer = found in *P. ponderosa* and nonhost conifers; NCV = nonhost conifer volatiles, found only in nonhost conifers; NAV = nonhost angiosperm volatiles, found only in angiosperms, NCAV = nonhost conifer and angiosperm volatiles, found in both angiosperms and nonhost conifers, Plant = general plant volatiles, found in the host, in other conifers, and in angiosperms; NHV = nonhost volatiles, compounds found in either or both nonhost angiosperms or nonhost conifers, but not in the host.

In total, 45 compounds were identified from host and nonhost trees that were antennally-active in one or both sexes of *D. brevicomis* in GC-EADs performed with either the natural extracts or commercially-available synthetic compounds. It was possible to classify these antennally-active compounds into six categories (Fig. 3). One compound (geraniol) was found exclusively in the primary host, *P. ponderosa*. Four compounds (*p*-cymene-8-ol, fenchyl alcohol, myrtenol, and terpinen-4-ol) were found in the host and one or more other conifers, but not in angiosperms. Five compounds (bornyl acetate, *exo*-brevicomin, *trans*-pinocarveol, *trans*-verbenol, and verbenone) were found in one or more nonhost conifers, but not in the host or in the angiosperms. Eight compounds [chalcogran, conophthorin, (*E*)-2- and (*Z*)-3-hexen-1-ol, (*E*)-2-hexenal, phenylethyl alcohol, salicylaldehyde, and guaiacol] were found exclusively in one or both of the angiosperms. Eight compounds (benzaldehyde, camphor, fenchone, hexanal, 1-hexanol, methyl salicylate, naphthalene, nonanal) were found in one or both of the angiosperms and one or more of the nonhost conifers, but not in the host conifer. Nineteen of the compounds were found in both the host and in either or both nonhost conifers or angiosperms.

Discussion

The ability of coniferophagous bark beetles to detect and respond to volatile compounds released from host and nonhost trees has been established (Byers 1995; Zhang & Schlyter 2004; Seybold *et al.* 2006). The antennal responses of *D. brevicomis* to volatiles from its host and to nine conifer and angiosperm nonhosts highlight the diversity of compounds and the complex interplay of olfactory signals encountered by foraging beetles in forests. Our results show that, while some compounds seem to be derived exclusively from hosts, nonhost conifers, or nonhost angiosperms, the delineation between such classes is not strongly demarcated. In our study, 43% of the antennally-active compounds were classified as general plant volatiles (Fig. 3), identified from extracts of the host, of the nonhost conifers, and/or of one or both of the angiosperms. However, 47% of the compounds were identified from a nonhost and 17% from both nonhost conifers and nonhost angiosperms. General conifer volatiles (emanating from the host and at least one nonhost conifer) comprised 9% of the antennally-active compounds that we observed. Only one (geraniol) of 47 antennally-active compounds was specific to *P. ponderosa*. Thus, a foraging coniferophagous bark beetle will likely encounter many compounds that emanate from its host as well as from nonhost conifers and/or angiosperms in its search area, but would likely not have enough information from any one compound to be able to make a foraging decision. The complexity of the olfactory landscape and related responses to mixtures of cues in other bark beetles has been suggested by previous trapping bioassays (Byers *et al.* 2000; Zhang & Schlyter 2003). The antennal responses of *D. brevicomis* observed in our study serve to highlight and explain the complex olfactory environment that would require the beetle to make flight direction and landing decisions based upon signals obtained from encountering a number of volatiles at once, or in close spatiotemporal proximity to each other.

In this environment, a foraging bark beetle would likely use various contextual cues to interpret chemical messages and would tailor its foraging decisions appropriately. These include visual cues such as bole reflectance (Strom *et al.* 1999; Strom & Goyer 2001; Campbell & Borden 2006), tactile or gustatory cues once in contact with the tree (Elkinton & Wood 1980), pheromone or allomone cues from con- or heterospecifics (Byers *et al.* 1984; Borden 1985; Byers

1985; Poland & Borden 1994; 1998a,b,c; Pureswaran *et al.* 2004), and other olfactory cues from intermixing plumes of host and nonhost volatiles. In the case of *D. brevicomis*, chemical cues have been shown to be more important than visual cues in disrupting aggregation to baited sources (Strom *et al.* 2001).

NAVs have been shown to disrupt the response of bark beetles, including *D. brevicomis* (Poland *et al.* 1998; Fettig *et al.* 2005), to aggregation pheromone cues in an additive and redundant manner. From the perspective of the researcher or pest manager who is seeking to find the best blend of compounds to evoke a preferred behavioral response this description seems to be partially correct. However, our study demonstrates that a foraging insect encounters an extremely large array of antennally-active compounds emanating from its host and a variety of nonhosts. Thus, from the perspective of the insect, the use of nonhost volatiles and other antiaggregants (or repellants) in pest management should be described as shifting the context in a realistic manner toward one in which a searching beetle would be reluctant to land on potential hosts for any appreciable amount of time, rather than simply attempting to overwhelm the insect with what is presumed to be a particular type of signal. Root's (1973) resource concentration hypothesis states that, in part, "herbivores are more likely to find and remain on hosts that are growing in dense or nearly pure stands." A diverse array of chemical signals from nonhost angiosperms, nonhost conifers, heterospecifics, and even conspecifics and host kairomones may disrupt bark beetle searching more than high doses of a single signal semiochemical or even a mixture of semiochemicals meant to represent one type of cue (e.g., a generic nonhost angiosperm) because diverse chemical signals represent a diverse stand to the foraging insect (Borden 1997). Because the odds of success for a searching beetle in a diverse stand are lower than in a more homogeneous stand of hosts (i.e., the extreme condition being a monoculture), a foraging beetle encountering a variety of semiochemicals may be induced to leave the area of perceived diversity instead of remaining and landing on candidate trees.

The presence of several compounds in plant volatiles that are also known to be produced by bark beetles as pheromone components, serves to increase the complexity of the foraging environment. Conophthorin, which is a pheromone of the white pine cone beetle, *Conophthorus coniperda* (Schwarz) and other bark beetles (Kohnle *et al.* 1992; Pierce *et al.* 1995; Birgersson *et al*. 1995; Dallara *et al*. 2000), is found in a number of angiosperms (Byers *et al*. 1998; Huber *et al*. 1999) including *P. tremuloides*. It has a disruptant effect on the aggregation behavior of a number of bark beetles (Byers *et al*. 1998; Huber *et al*. 1999; 2000a), but perhaps not on *D. brevicomis* (Fettig *et al*. 2005). At least one of either *exo*-brevicomin, *trans*-verbenol, verbenone, or myrtenal were present in the volatiles of all of the conifers in our survey. In the case of the latter three, when emanating from conifers, they may simply be products of autoxidation (Moore *et al*. 1956) or microbial metabolism of α-pinene (Byers *et al*. 1989). Verbenone is an anti-aggregation pheromone of a number of pine-infesting bark beetles including *D. brevicomis* (Tilden & Bedard 1988; Bertram & Payne 1994). *trans*-Verbenol and *exo*-brevicomin, are also found in volatiles associated with *D. brevicomis* (Byers & Wood 1980; Byers 1983; Byers *et al.* 1984) and are likely produced by the beetles or their symbionts. Myrtenal may also be a pheromone component in *Dendroctonus* spp. as it is known to be present in the gut of *D. frontalis*, is detected by their antennae, and has behavioral effects at high release rates (Sullivan 2005).

exo-Brevicomin is an aggregation pheromone component for *D. brevicomis* (Bedard *et al*. 1969; 1980b) and was identified from and detected by *D. brevicomis* antennae only in *A. concolor*. Notably, it is also an aggregation pheromone component of the western balsam bark beetle, *Dryocoetes confusus* Swaine, which infests grand fir, *Abies grandis* (Douglas ex D. Don) Lindley, and subalpine fir, *A. lasiocarpa* (Hook.) Nutt. This raises the possibility that some *Abies*-infesting bark beetles that were capable of detecting and responding behaviorally to the host volatile as a kairomone later evolved the capacity to produce the compound as an aggregation pheromone that induces a positive response toward the source in conspecifics. On the other hand, it is possible that host trees have, in some cases, evolved the capability of producing bark beetle pheromones components as a defense that can be used to manipulate beetle behavior.

trans-Verbenol is an antiaggregant for *D. brevicomis* (Bedard *et al.* 1980a; Byers *et al.* 1984). It is produced by the insect via conversion of enantiomers of α-pinene in the host to the same enantiomers of *trans*-verbenol (Byers 1983). Its presence in the volatiles of the nonhost conifer *P. menziesii* suggests a potential role of a nonhost in the evolution of pheromone biosynthetic capabilities and behavioral response to this compound by *D. brevicomis*. Interestingly, it is also a known antiaggregant of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins (Pureswaran & Borden 2004), which infests *P. menziesii*.

Verbenone was not detected by FID or EAD in the volatiles of either its primary host, *P. ponderosa* or in *P. contorta murrayana*, both of which are also hosts of the mountain pine beetle, *D. ponderosae* Hopkins. Verbenone was, however, present in detectable quantities in *P. jeffreyi* and *P. lambertiana*, and has been reported from *P. radiata* D. Don (Cool & Zavarin 1992; Dallara *et al*. 2000) and *P. sylvestris* (Byers *et al*. 1989). The occurrence of verbenone in pines may suggest the possibility of some role of an ancestral host in the evolution of the ability of pine-infesting *Dendroctonus* spp. to detect or synthesize this compound. Verbenone was present in *P. jeffreyi*, a pine that is not utilized by either *D. brevicomis* or *D. ponderosae*, indicating that this compound should be considered both a NHV and an antiaggregation pheromone, depending upon the context in which the foraging beetle detects it.

There is some existing evidence that bark and ambrosia beetles display positive, or at least not negative chemotaxis, to mixtures of nonhost volatiles and pheromone components (Deglow & Borden 1998a,b; Huber *et al.* 2001). Huber *et al.* (2001) hypothesized that bark beetles foraging for host material that was shrouded in leafy plants may often encounter mixtures of green leaf volatiles (GLVs) and conspecific aggregation pheromone components. Since it would be beneficial for the foraging beetle to orient towards the shrouded host, it may display positive chemotaxis or may

not respond to GLVs when mixed with pheromone components. In a similar manner, if bark beetle responses to nonhost volatiles and pheromone components are viewed in the contextual manner, then it is possible that some compounds that are generally thought of as attractant pheromone components, but which are also nonhost volatiles, may act to disrupt bark beetle aggregation when presented in the context of other nonhost volatiles. This possibility has not been tested in a directed manner, as most assays with nonhost volatiles include aggregation pheromone components, either in the trap with plant volatiles, or applied to host trees. In at least one instance, however, when no aggregation pheromone components were used in conjunction with NHVs and verbenone, *D. ponderosae* was not disrupted from attacking host trees, although a number of prior experiments, which had utilized aggregation pheromone components, showed very strong inhibitory effects (Huber & Borden 2001b). Failures of NHVs to disrupt bark beetle aggregation in pest management settings may, then, be due to a lack of certain pheromone components in the mixture. In addition, the successful use of NHVs, when paired with verbenone against *D. brevicomis* and *D. ponderosae*, as compared to verbenone on its own, may be ascribed to the creation of a more realistic nonhost signaling context rather than to the summed contribution of negative signals from different contexts. Thus, while verbenone has been shown in some trials to protect pines from bark beetle attack (Lindgren *et al.* 1989; Shore *et al.* 1992; Shea *et al.* 1992; Lindgren & Borden 1993; Progar 2003) some failures (Gibson *et al.* 1991; Shea *et al.* 1992; Progar 2003) may not simply be due to photoisomerization of the compound to an inactive form as has been suggested (Kostyk *et al.* 1993), but also due to a lack of creation of a realistic context for foraging insects. That is, synthetic verbenone alone, deployed without other beetle-derived or nonhost cues in appropriate quantities, may not always provide foraging beetles with the desired misinformation about the stand in which they are foraging. Further work in which low amounts of compounds that are both beetle pheromone components, such as frontalin (Huber *et al.* 1999), *trans*-verbenol, and *exo*-brevicomin (this study), are released in conjunction with verbenone and other NAVs should be conducted to test this hypothesis.

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