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GC-EAD responses to semiochemicals by eight beetles in the subcortical community associated with Monterey pine trees in coastal California: similarities and disparities across three trophic levels

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Summary. Antennae of six sympatric bark and ambrosia beetles (Scolytidae), *Dendroctonus valens* LeConte, *Gnathotrichus retusus* (LeConte), *Hylastes tenuis* Eichhoff, *Ips mexicanus* (Hopkins), *Ips plastographus maritimus* Lanier, and *Pseudohylesinus sericeus* (Mannerheim), and two scolytid predators, *Enoclerus sphegeus* (F.) (Cleridae) and *Lascontonus tuberculatus* Kraus (Colydiidae), were analyzed by gas chromatographic-electroantennographic detection (GC-EAD) for their responses to synthetic *Ips* spp. pheromone components, and host and nonhost volatiles. The beetles emerged from cut logs of pitch canker-infected Monterey pine trees, *Pinus radiata* D. Don. There were significant disparities in EAD response patterns to the hemiterpene and monoterpene alcohol pheromone components that are typically produced by *Ips* spp. Antennae of *I. p. maritimus* responded strongly to (±)-ipsdienol, (±)-ipsenol, amitinol, and lanierone; antennae of *I. mexicanus* responded strongly to (1*S*,2*S*)-(–)-*cis*-verbenol, with weaker responses to (±)-ipsdienol, (±)-ipsenol, and amitinol; antennae of *H. tenuis* responded to (1*S*,2*R*)-(–)-*trans*-verbenol, with less pronounced responses to (–)-*cis*-verbenol and 2-methyl-3-buten-2-ol; and antennae of *D. valens*, *G. retusus*, and *P. sericeus* generally responded to all *Ips* spp. pheromone components except 2-methyl-3-buten-2-ol (*D. valens* and *G. retusus*) and *E*-myrcenol (*G. retusus* and *P. sericeus*). *Ips mexicanus* responded only to the (–)-enantiomers of ipsenol and ipsdienol, whereas *I. p. maritimus* responded to (–)-ipsenol, but to both the (+)- and (–)-enantiomers of ipsdienol. The antennae of the two predaceous insects (*E. sphegeus* and *L. tuberculatus*) responded to a range of the *Ips* spp. pheromone components. Host monoterpenes elicited no antennal responses from *E. sphegeus*, *G. retusus*, *H. tenuis*, and *I. mexicanus*, but several monoterpenes elicited various levels of responses from *D. valens* and *I. p. maritimus* antennae. Interestingly, antennae of female *D. valens* responded to (–), but not (+)-limonene. α- and β-Pinene elicited weak responses from *L. tuberculatus* antennae. EAD responses to selected nonhost volatiles were almost identical among the six scolytid species, with *trans*-conophthorin eliciting the strongest response in most cases, followed by three C_6-alcohols and two C_8-alcohols. The antennal responses by most of these species to linalool or geranylacetone were very weak; (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, and benzyl alcohol elicited almost no response. The response pattern of *P. sericeus* to nonhost volatiles differed slightly from the rest of the scolytids: a strong response to linalool, weaker response to the C_8-alcohols. The two predaceous Coleoptera generally had weak, but detectable, responses to nonhost volatiles, except for a relatively strong response to *trans*-conophthorin by *L. tuberculatus.* No notable differences in EAD responses were observed between males and females of the two *Ips* spp. Our results provide an electrophysiological baseline for future efforts to identify attractive and repellent semiochemicals (aggregation pheromones, host kairomones, or nonhost interruptants) for this guild of scolytids and their key predators that are associated with moribund and pitch canker-infected *P. radiata.*

Key words. Ambrosia beetle – bark beetle – chemical ecology – Cleridae – Coleoptera – Colydiidae – *Dendroctonus valens* – electrophysiology – *Enoclerus sphegeus* – host selection – *Ips mexicanus* – *Ips plastographus maritimus* – kairomone – *Lasconotus tuberculatus* – monoterpenes – pheromone – *Pinus radiata* – Scolytidae – subcortical insects

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Introduction

Monterey pine, *Pinus radiata* D. Don., is native to coastal California in three very limited areas in Monterey, San Luis Obispo, and Santa Cruz Counties, and on Guadalupe and Cedros Islands off of the west coast of Baja California, Mexico. In contrast to its restricted native range, *P. radiata* is one of the most widely planted pine species in the world, especially in the warm temperate regions such as Australia, New Zealand, Spain, Africa, and South America (Lavery & Mead 1998). Since the late 1980s, native stands of *P. radiata* in California have been severely impacted by the fungus *Fusarium circinatum* (= *F. subglutinans* f.sp. *pini*), a causal agent of pitch canker disease (Storer *et al.* 1997). Several bark beetles (Coleoptera: Scolytidae) (*sensu* Wood 2007), *Conophthorus radiatae* Hopkins, *Ips mexicanus* (Hopkins), *I. paraconfusus* Lanier, *I. plastographus maritimus* Lanier, *Pityophthorus carmeli* Swaine, *P. nitidulus* Mannerheim, and *P. setosus* Blackman, have been shown to be associated with the fungus and to visit and infest uninfected trees (Fox *et al.* 1991; Storer *et al.* 2004; Erbilgin *et al.* 2008). Other sympatric scolytids, such as *Dendroctonus valens* LeConte (a lower stem-infesting bark beetle), *Gnathotrichus retusus* (LeConte) (a stem-colonizing ambrosia beetle), *Hylastes tenuis* Eichhoff (a root collar- and root-feeding bark beetle), and *Pseudohylesinus sericeus* (Mannerheim) (= *pini* Wood) (an early arriving species on the stem of freshly felled trees) have also been found to colonize pitch canker-infected *P. radiata* along the California coast (Erbilgin, pers. obs.). Coleoptera that prey on the scolytids, e.g., the redbellied clerid, *Enoclerus sphegeus* (F.) (Cleridae), and the cylindrical bark beetle, *Lascontonus tuberculatus* Kraus (Colydiidae), are also an integral part of this forest insect community. All insect species associated with *P. radiata* in this area of California, and especially the subcortical taxa, have been exhaustively cataloged (Ohmart 1981; Ohmart & Voigt 1982). Prevention of establishment of bark beetle vectors in high value pine stands with attractive and interruptive semiochemicals in a push-pull fashion (Borden 1997) might be an efficacious management tool in pitch canker integrated pest management.

Basic knowledge of the chemical ecology of these subcortical beetle species that colonize pitch canker-infected *P. radiata* in coastal California is very limited. The aggregation pheromones (if present) of *D. valens*, *H. tenuis*, *I. mexicanus*, *I. p. maritimus,* and *P. sericeus* have not yet been determined; the aggregation pheromone of *G. retusus* is known from work in another ecosystem (Borden *et al.* 1980). Similarly, what is known of the chemical ecology of *E. sphegeus* has also been learned from other forest ecosystems (Seybold *et al.* 1992; Lindgren & Miller 2002); to our knowledge the chemical ecology of *L. tuberculatus* has not been investigated. No EAD response data to semiochemicals are available for any of these beetle species; however, nonhost green leaf volatiles (mainly C_6-alcohols) have been reported to disrupt the flight response of *G. retusus* to pheromone-baited traps (Deglow & Borden 1998).

We used gas chromatographic-electroantennographic detection (GC-EAD) to examine the antennal responses of eight subcortical species of Coleoptera that occur in moribund *P. radiata* in coastal California. Semiochemicals tested included synthetic *Ips* spp. pheromone components, host volatiles, and angiosperm nonhost volatiles. Our hypotheses were H_1: there would be significant differences in the EAD response patterns to the *Ips* spp. pheromone components among the eight sympatric beetle species; H_2: there would be no differences in the EAD response patterns to common host monoterpenes; and H_3: there would be no differences in the EAD response patterns to the nonhost volatiles. No efforts (or intentions) were made to identify the pheromone or other semiochemical systems of these beetles with natural materials in the current study; however, our results will provide an electrophysiological baseline at the peripheral level for future behavioral assays that may lead to identification of pheromones, host kairomones, and nonhost repellents.

Materials and Methods

Collection of live insects

The beetles were reared from cut logs collected from several pitch canker-infected *P. radiata* trees (cut 15–17 May 2006 near Spanish Bay, Monterey Co., California, 36°36′ N, 121°54′ W and 14 May 2008 near the Pebble Beach Corporation Yard, Monterey Co., California, 36°35.096′ N, 121°55.793′ W). Beetles were reared by placing the logs in emergence boxes as described in Browne (1972). Emerged adults were stored in glass vials with moist tissue paper at 4°C until they were used for GC-EAD recordings. The sexes of *D. valens* were separated by the presence of the stridulatory organ on the abdominal tip of the males (Lyon 1958). The sexes of *I. mexicanus* and *I. p. maritimus* were separated as described in Lanier & Cameron (1969). Since the separation of the sexes of *I. mexicanus* cannot be achieved with 100% accuracy by using external morphological characters, final confirmation was made by dissection and observation of the genitalia. Because the generic status of *I. mexicanus* is unresolved in the literature (Cognato 2000; Wood 2007), we opted conservatively to use the generic nomenclature from the most recent world catalog of the family (Wood & Bright 1992). The sexes of *E. sphegeus*, *L. tuberculatus*, and *P. sericeus* were also deteremined by dissection and observation of the genitalia. The sexes of *G. retusus* and *H. tenuis* were not separated. Voucher specimens of *D. valens, E. sphegeus*, *G. retusus*, the *Ips* spp., *L. tuberculatus* and *P. sericeus* were deposited in the California Academy of Sciences, San Francisco, California, USA.

Semiochemicals

Synthetic semiochemicals were obtained from various commercial and noncommercial sources. 1) *Ips* spp. pheromone components: (±)-ipsenol (95%), (±)-ipsdienol (95%), and (1*S*)-(–)-verbenone (99%) (Bedoukian Research Inc., Danbury, CT, USA); (1*S*,2*S*)-(–)-*cis*-verbenol (98%, Borregaard, Sarpsborg, Norway); (1*S*,2*R*)-(–)-*trans*-verbenol (>98%; IOCB, Prague, Czech Republic); amitinol (98%, W. Francke, Universität Hamburg, Hamburg, Germany); (*E*)-myrcenol (95.2%, SciTech, Prague, Czech Republic); 2-methyl-3-buten-2-ol (97%; Acros, Morris Plains, NJ, USA); 3-methyl-3-buten-1-ol (>97%, Sigma-Aldrich, Milwaukee, WI, USA); and lanierone (commercial lure

from Pherotech International, Inc., Delta, BC, Canada). 2) Host monoterpenes included several major compounds previously identified from tissues of *P. radiata* (Mateus *et al.* 1997): (±)-α-pinene (98%), (–)-β-pinene (99%), (+)-Δ^3-carene (>90%), *p*-cymene (99%), and (*R*)-(+)-limonene 97% (Sigma-Aldrich), and β-myrcene (95%), (*S*)-(–)-limonene (96%), α-terpinene (95%), a mixture of *trans*/*cis*-ocimenes (>90%), and terpinolene (>95%) (Fluka, St. Louis, MO, USA). 3) Nonhost volatiles: (±)-*trans*-conophthorin (87%; Pherotech International); (*E*)-2-hexenal (99%), (*Z*)-3-hexenyl acetate (>98%), 1-hexanol (98%), (*Z*)-3-hexen-1-ol (98%), (*E*)-2-hexen-1-ol (97%), benzyl alcohol (99%), and geranylacetone (95%) (Sigma-Aldrich); and (±)-3-octanol (99%) and (±)-1-octen-3-ol (98%) (Acros). (*R*)-(–)-Linalool (97%, Acros) was also tested, but it may be classified as both a host and a nonhost volatile. It is present in the essential oils of a wide range of plants (Merck & Co. 1996), including pines (Mirov 1961; Mirov *et al.* 1962; Zavarin *et al.* 1971; Mateus *et al.* 1997). Linalool is likely to be present in the xylem oleoresin of most pines (LG Cool, University of California at Berkeley, personal communication).

Gas chromatographic-electroantennographic detection (GC-EAD) analyses

Synthetic mixtures [ca. 100–200 ng/µl each compound in hexane (98.5%, Sigma-Aldrich)] of *Ips* spp. pheromone components, host monoterpenes, and nonhost (green leaf and angiosperm bark) volatiles were injected (1–3 µl) splitless into a Varian CP-3800 GC equipped with a polar column (HP-INNOWax; 30 m × 0.53 mm × 1.0 µm film thickness; Agilent Technologies, Wilmington, DE, USA) and a 1:1 effluent splitter that allowed simultaneous flame ionization detection (FID) and EAD of the separated volatile compounds. These synthetic mixtures were similar to those described in Zhang *et al.* (2001, 2003, 2007) for Eurasian conifer bark beetles. Helium was used as the carrier gas, and the injector and detector temperatures were 250°C and 300°C, respectively. Column temperature was 50°C for 1 min, rising to 240°C at 10°C/min, and then held for 10 min. The outlet for the EAD was held in a humidified air stream flowing at 0.5 m/sec over the antennal preparation. A glass capillary indifferent electrode filled with Beadle-Ephrussi Ringer solution (128 mM NaCl, 4.69 mM KCl, and 1.97 mM $CaCl_2$), and grounded *via* a silver wire, was inserted into the severed beetle's head with the antennae. A similar recording electrode connected to a high-impedance DC amplifier with automatic baseline drift compensation was placed in contact with the distal end of the antennal club (Zhang *et al.* 2000). The signal was stored and analyzed on a PC equipped with a serial IDAC interface box and appropriate software (EAD ver. 2.5 from Synthech, Hilversum, The Netherlands). The species were analyzed in two batches (7 to 12 September 2006: *G. retusus, H. tenuis, I. mexicanus*, and *I. p. maritimus*; 13 to 17 June 2008: *D. valens, E. sphegeus, L. tuberculatus*, and *P. sericeus*), and the results are presented by batch. For each synthetic mixture, electroantennograms were recorded from more than three antennae of each species, with the exception of *E. sphegeus* where recordings were made from two antennae. Lanierone was not available to us as a neat material or in solution form, so GC-EAD analysis was conducted with SPME (CAR/PDMS, 75 µm; Supelco, Bellefonte, PA, USA) samples from a used bubble cap dispenser (Pherotech International product L1–3300/000). The lanierone was present in the release device as a solution (1,3-butanediol as solvent; 0.5 ml in a 8-ml glass vial and headspace was sampled for 5 min) and was tested only against the antennae of *I. p. maritimus.* The SPME headspace sample was also analyzed by GC-MS on an HP 6890 GC series coupled with an HP 5973 Mass Selective Detector with the same type of GC column and conditions as described above.

GC-EAD analysis by enantioselective gas chromatography

Antennal responses of *I. mexicanus* and *I. p. maritimus* were also analyzed with a synthetic mixture of ipsenol, amitinol, and ipsdienol (100 ng/µl each in hexane) separated by GC with an enantioselective stationary phase. The enantiomers in the solution were separated by injecting the sample splitless on the Varian CP-3800 GC equipped with an Rt-bDEXm™ column (30 m × 0.25 mm × 0.25 µm film thickness; Restek, Bellefonte, PA) and a 1:1 effluent splitter. The antennal preparation and EAD recording system were as described above. Helium was again used as the carrier gas, and the injector/detector temperatures were both 230°C. Column temperature was 80°C for 1 min and rose to 200°C at 2°C/min. Elution order of the (–)- or (+)-enantiomers of ipsenol and ipsdienol [(–)- eluted before (+)- for both compounds] were determined as described in Seybold (1992) and Seybold *et al.* (1992, 1995a,b), for which columns with similar enantioselective stationary phases were used. The separation factors (α-values) were 1.01 for the enantiomers of both ipsenol and ipsdienol, calculated based on their corresponding retention times adjusted by the retention time of pentane.

Results

No notable differences in EAD responses were detected between the antennae of males and females of the two *Ips* spp. *Ips p. maritimus* antennae responded strongly to ipsdienol, ipsenol, and amitinol (Fig. 1A). The responses to 2-methyl-3-buten-2-ol, (–)-*cis*- and (–)-*trans*-verbenol, and (–)-verbenone were evident, but very weak; no antennal activity was detected in response to 3-methyl-3-buten-1-ol or *E*-myrcenol at the dosage tested. In *I. mexicanus*, the strongest EAD response was to *cis*-verbenol, followed by ipsdienol, ipsenol, and amitinol. Antennal responses to *trans*-verbenol and verbenone were very weak, but detectable; no antennal activity was detected in response to the two methylbutenols or to *E*-myrcenol at the dosage tested (Fig. 1A). *trans*-Verbenol elicited the strongest EAD response from the antennae of *H. tenuis*, followed by *cis*-verbenol and 2-methyl-3-buten-2-ol. No antennal responses from *H. tenuis* were recorded to the other *Ips* spp. pheromone components tested. Antennae of *G. retusus* responded to all compounds tested except for 2-methyl-3-buten-2-ol and *E*-myrcenol (Fig. 1A). *Dendroctonus valens* responded to all compounds tested except 2-methyl-3-buten-2-ol (Fig. 1B). The strongest responses were recorded to *cis*- and *trans*-verbenol, ipsenol, ipsdienol, and verbenone. *Pseudohylesinus sericeus* showed weak responses to all *Ips* spp. pheromone components with the exception of *E*-myrcenol, to which there was no response (Fig. 1B). The strongest responses were to 2-methyl-3-buten-2-ol and *cis*-verbenol. Antennae of the predators, *E. sphegeus* and *L. tuberculatus*, responded most strongly to ipsenol (both species), *cis*- and *trans*-verbenol (*E. sphegeus*), ipsdienol (*L. tuberculatus*), and verbenone (both species) (Fig. 1B). There may have been a weak antennal response by *E. sphegeus* to ipsdienol, but the signal appears as a shoulder with that of *trans*-verbenol in the output (Fig. 1B). There was a weak response to amitinol by *L. tuberculatus. Ips p. maritimus* antennae showed no EAD activity to the solvent (1,3-butanediol) from the used lanierone commercial lure, but were strongly responsive to lanierone and a trace amount of ipsdienol (identified by GC-MS) (Fig. 2), which was probably a contaminant from the field trapping experiment with ipsdienol lures.

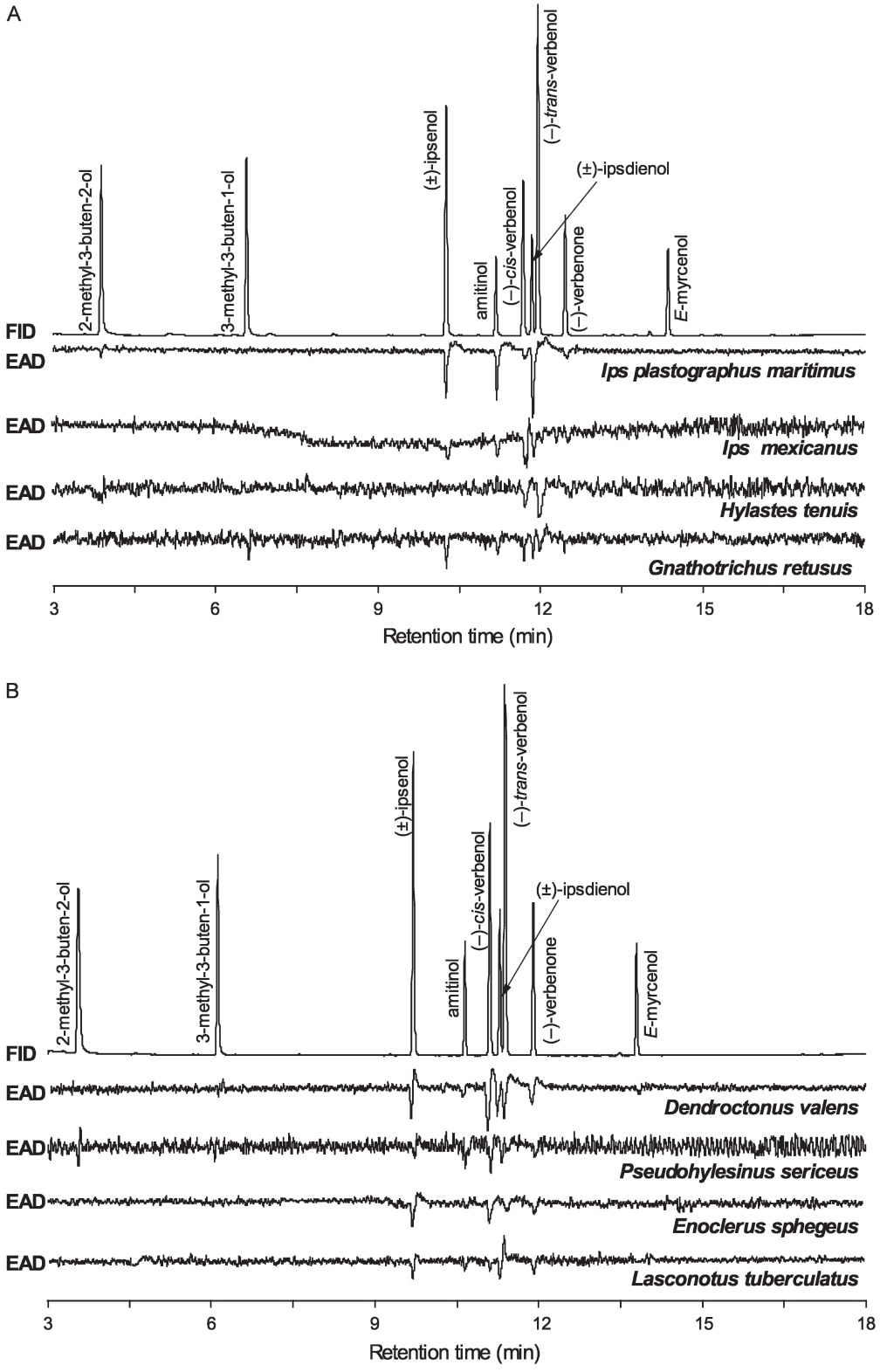


Fig. 1. GC-EAD responses of the antennae of (**A**) four scolytid species on 7 to 12 September 2006 and (**B**) two scolytid species, one clerid species, and one colydiid species (all Coleoptera) on 13 to 17 June 2008 to a synthetic mixture of *Ips* spp. pheromone compounds (ca. 125 ng each compound) separated with an HP-INNOWax 30 m x 0.53 mm capillary GC column. All Coleoptera emerged from cut logs of Monterey pine, *Pinus radiata* D. Don. All EAD responses in panel (**B**) were recorded from females.

GC-EAD responses to the enantiomers of selected *Ips* spp. pheromone components indicated that *I. p. maritimus* antennae responded to (–)-ipsenol, and to both (–)- and (+)-ipsdienol, whereas *I. mexicanus* antennae only responded to the (–)-enantiomers of ipsenol and ipsdienol (Fig. 3). As in the prior analysis on the HP-INNOWAX stationary phase (Fig. 1A), antennae of both species responded to amitinol (Fig. 3).

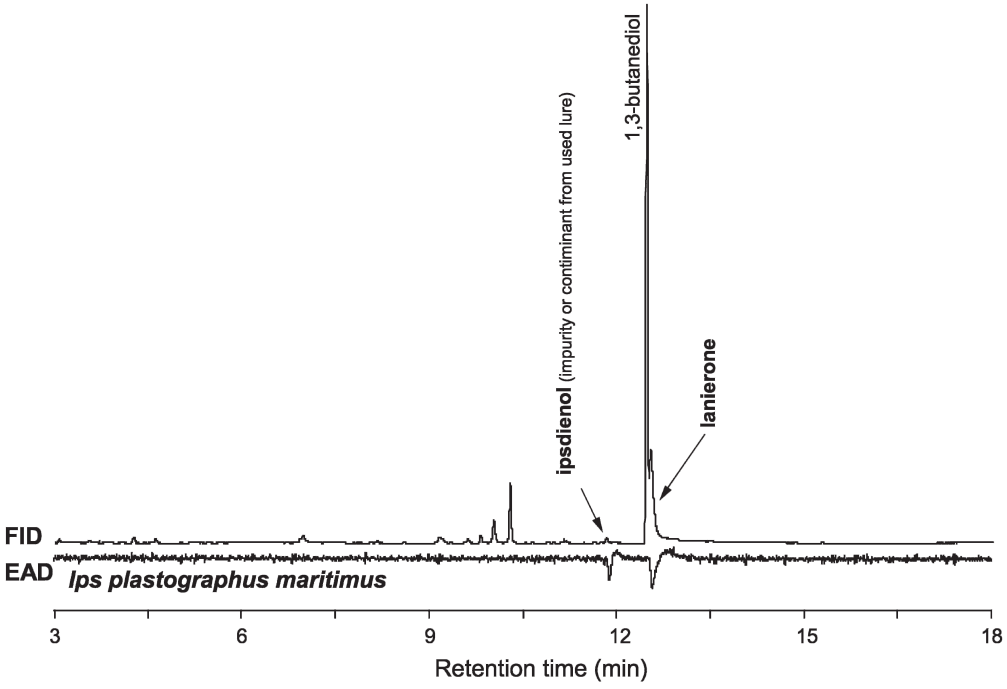


Fig. 2. GC-EAD responses of the antennae of *Ips plastographus maritimus* to a SPME (CAR/PDMS) sample from the headspace of a used lanierone commercial lure (1,3-butanediol as solvent) separated with an HP-INNOWax 30 m x 0.53 mm capillary GC column.

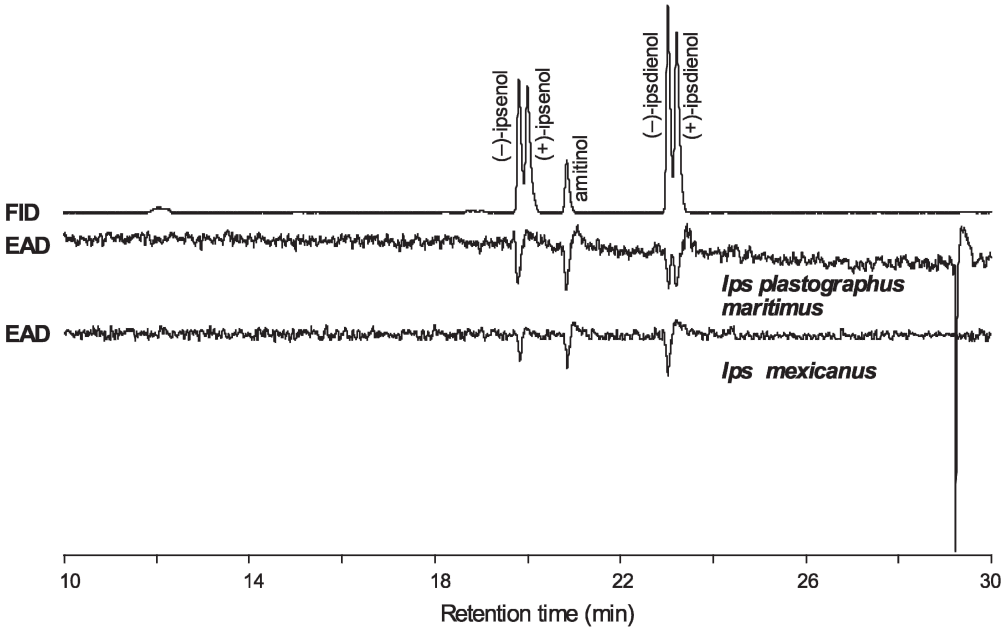
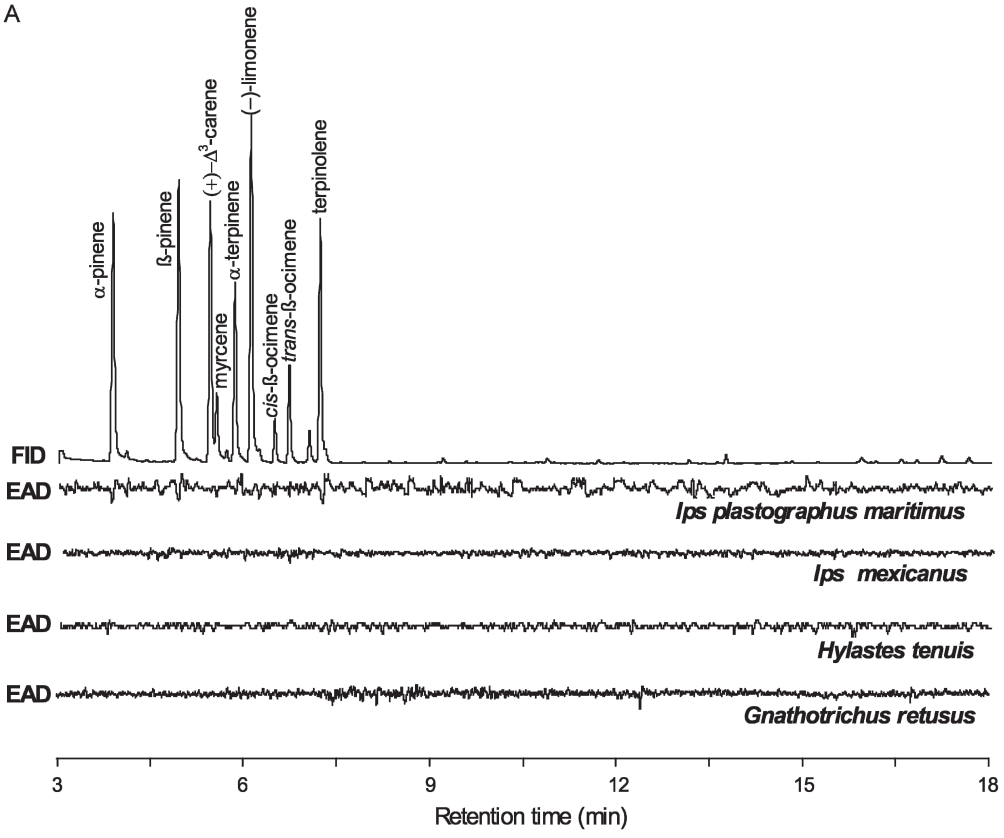


Fig. 3. GC-EAD responses of antennae of *Ips plastographus maritimus* and *Ips mexicanus* to a synthetic mixture of racemic ipsenol, amitinol, and racemic ipsdienol (ca. 50 ng each compound), whose enantiomers were separated with a Rt-bDEXm™ 30 m × 0.25 mm capillary GC column. Amitinol is achiral.

Weak (but repeatable) antennal responses were recorded to α- and β-pinene, and terpinolene from *I. p. maritimus* at the dosage tested (ca. 300 ng) (Fig. 4A). Similar weak antennal responses were recorded to α- and β-pinene, and (+)-Δ^3-carene from female *D. valens*, whereas β-myrcene, α-terpinene, (–)-, but not (+)-limonene, and terpinolene elicited strong EAD responses (Fig. 4B). No EAD responses to monoterpenes were detected from antennae of any of the other species at the same stimulant dosage, with the exception of very minor responses to α- and β-pinene by male *L. tuberculatus* (Fig. 4B). Antennal responses of *P. sericeus* to monoterpenes were not recorded because we did not have enough specimens for the test.

EAD responses to the nonhost volatiles and linalool were almost identical among the six scolytid beetle species (Fig. 5A,B). *trans*-Conophthorin elicited the strongest response in most cases, followed by the three green leaf alcohols (C_6-alcohols), and the two C_8-alcohols. Female *P. sericeus* was somewhat exceptional because it had



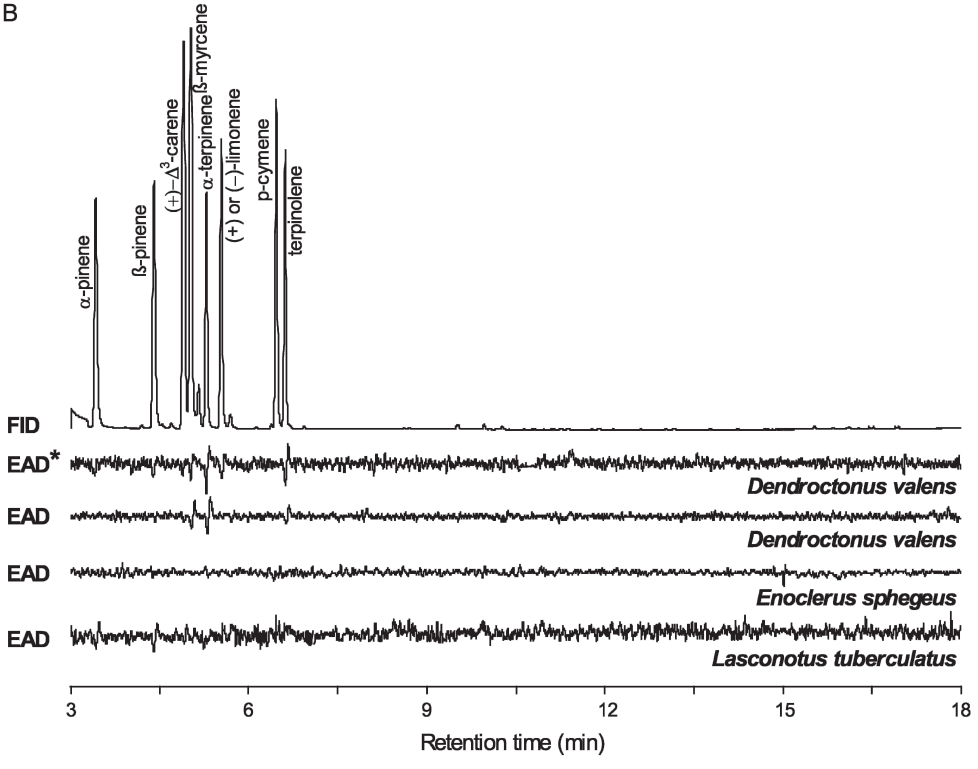


Fig. 4. GC-EAD responses of the antennae of (**A**) four scolytid species on 7 to 12 September 2006 and (**B**) one scolytid species, one clerid species, and one colydiid species (all Coleoptera) on 13 to 17 June 2008 to a synthetic mixture of host monoterpenes (ca. 300 ng each compound) separated with an HP-INNOWax 30 m x 0.53 mm capillary GC column. The monoterpene mixtures tested on each batch of insects were the same with a few exceptions. In the first batch (**A**) we tested (–)-limonene, but with *D. valens* in the second batch (**B**), we tested the mixture first with (–)-limonene* and then again with (+)-limonene. The responses of all other taxa in the second batch were tested against (+)-limonene. Further, *cis*- and *trans*-β-ocimene included in the mixture that was tested on the first batch (**A**) were replaced with *p*-cymene in the mixture that was tested on the second batch (**B**). All Coleoptera emerged from cut logs of Monterey pine, *Pinus radiata* D. Don. All EAD responses in panel (**B**) were recorded from females with the exception of *L. tuberculatus*, which was a male.

weak antennal responses to the C_8-alcohols, but a strong response to linalool (Fig. 5B). Otherwise, the antennal responses to linalool or geranylacetone were very weak, and not repeatable for all species. (*E*)-2-Hexenal, (*Z*)-3-hexenyl acetate, and benzyl alcohol were EAD inactive for the scolytids at the dosage tested (Fig. 5A,B). Antennal responses of the predators to nonhost volatiles were all relatively weak (Fig. 5B). Female *E. sphegeus* responded most strongly to the C_6-alcohols, linalool, and (*E*)-2-hexenal with only a minor response to *trans*-conophthorin. Male *L. tuberculatus* responded strongly only to *trans*-conophthorin, with weaker responses to 1-octen-3-ol and linalool (Fig. 5B).

Discussion

The subcortical species of Scolytidae that we analyzed in this study included three that typically colonize the phloem of the main stem and larger branches of *P. radiata* (*Ips* spp. and *P. sericeus*); one that colonizes the phloem of the lower portion of the main stem and extends its galleries below the soil line (*D. valens*); one that colonizes the phloem at the root collar or below the soil line (*H. tenuis*); and one that colonizes the xylem of the main stem (*G. retusus*) (Wood 1982). The two predators (*E. sphegeus* and *L. tuberculatus*) occur as larvae (both species) and adults (*L. tuberculatus*) in the galleries of the bark beetles (Ohmart 1981). All of these taxa may also co-occur in fallen stem sections, as *H. tenuis* will likely colonize the underside of sections that are in contact with the soil.

Few analyses by EAG or GC-EAD have been reported for a guild of subcortical beetles from one host as a homogenous complex (e.g., Smith *et al*. 1988; Huber *et al*. 2000, but in the latter case only for two scolytid species from the same host). No previous investigations have addressed the antennal responses of both herbivores and carnivores as a subcortical ensemble. Although the arrival patterns on freshly felled *P. radiata* by *I. p. maritimus* and associated insects have been described (Ohmart & Voigt 1982), and the preferential twig beetle, *Pityophthorus* spp., colonization of *F. circinatum*-infected branches of *P. radiata* has been reported (Bonello *et al.* 2001), the patterns of spatial and temporal colonization of *P. radiata* in relationship to *F. circinatum* have not been clearly described for the ensemble of subcortical species of Coleoptera noted above in our study. Furthermore, olfactory mechanisms involved in the host selection and semiochemical interactions of these subcortical beetles with or without involvement of *F. circinatum* have not been studied yet.

In the current study, *I. p. maritimus* antennae responded strongly to ipsdienol, ipsenol, and amitinol, which are frequently occurring aggregation pheromone components of *Ips* bark beetles (Seybold & Vanderwel 2003), whereas only weak or no responses were detected to *cis*- or *trans*-verbenol (Fig. 1A). Seybold (1992) identified 89- to 95%-(+)-ipsdienol from Porapak-trapped volatiles from *P. radiata* logs artificially colonized by *I. p. maritimus*, but its attractiveness in the field has not yet been demonstrated (Warren *et al.* 1996). Interestingly, lanierone, a pheromone component of *I. pini* (eastern North American populations) (Teale *et al.* 1991) and attractive in the field to *Ips integer* (Eichhoff) (Miller *et al.* 1997) and various *Ips* spp. from southeastern North America (Birgersson *et al.* 1995; Miller *et al.* 2005), also elicited strong EAD-activity from antennae of *I. p. maritimus* (Fig. 2). However, it is not known whether *I. p. maritimus* males produce this component or other EAD-active compounds as part of their pheromone system. The EAD response pattern of *I. mexicanus* differed from *I. p. maritimus.* Antennae of *I. mexicanus* responded more strongly to (–)-*cis*-verbenol than to ipsenol or ipsdienol (Fig. 1A). The aggregation pheromone system of *I. mexicanus* has not been identified yet, but Seybold (1992) identified 90%-(–)-ipsdienol from coastal (i.e., *P. radiata*) populations and both (–)-ipsenol and 90%-(–)-ipsdienol from montane (i.e., *P. contorta murrayana*) populations in California. It appears that lanierone and *cis*-verbenol play important roles in the flight attraction of *I. p. maritimus* and *I. mexicanus*, respectively, in the field (Erbilgin, Wood, Seybold, unpublished data).

GC-EAD analysis with an enantioselective stationary phase column indicated that the antennae of *I. mexicanus* responded only to the (–)-enantiomers of ipsenol and ipsdienol, whereas the antennae of *I. p. maritimus* responded to (–)-ipsenol, and to both the (–)- and (+)-enantiomers of ipsdienol (Fig. 3). If ipsenol and ipsdienol are part of the aggregation system for *I. mexicanus*, then inexpensive racemic blends of these two compounds can likely be used for commercial lure development. However, in the case of *I. p. maritimus*, the EAD-active "unnatural" (–)-enantiomer of ipsdienol may antagonize attraction. An enantioselective EAD response to (–)-ipsenol, but lack of enantioselectivity in response to ipsdienol was also recorded from *Ips confusus* (LeConte) (Seybold *et al*. 2004; http://www.chemecol.org/meetings/Abstracts%20Ottawa%202004.pdf).

Little is known about the potential for pheromones with *D. valens*, *H. tenuis*, and *P. sericeus.* The relatively strong antennal response of *D. valens* to most of the *Ips* spp. pheromone components, especially ipsenol, may reflect allomonal communication among *D. valens*, the two *Ips* noted above, and/or other *Ips* spp. that occur in sympatry with *D. valens* in North America. However, responses to ipsdienol (often produced by male *Dendroctonus* spp., Seybold & Vanderwel 2003), and *cis*- and *trans*-verbenol and verbenone (Hughes 1973) may indicate pheromonal or allomonal communication. Verbenone interrupts the flight response of *D. valens* to host attractants in the field (Rappaport *et al.* 2001). In contrast to the *Ips* spp., antennae of *H. tenuis* responded strongly to (–)-*cis*- and -*trans*-verbenol, weakly to 2-methyl-3-buten-2-ol, and were unresponsive to the *Ips* spp. pheromone components (±)-ipsenol and (±)-ipsdienol (Fig. 1A). It will be interesting to test whether *H. tenuis*

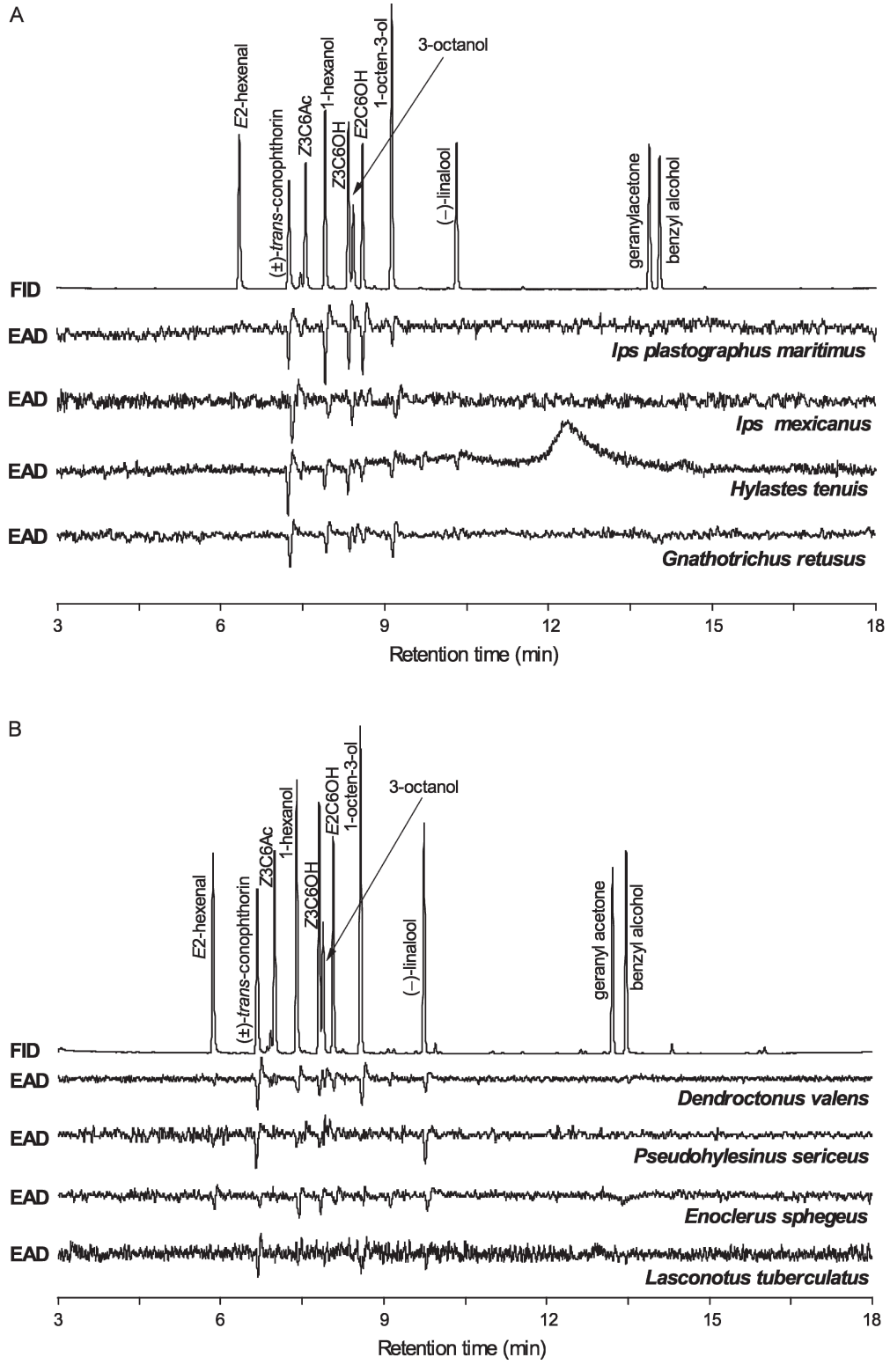


Fig. 5. GC-EAD responses of the antennae of (**A**) four scolytid species on 7 to 12 September 2006 and (**B**) two scolytid species, one clerid species, and one colydiid species (all Coleoptera) on 13 to 17 June 2008 to a synthetic mixture of angiosperm nonhost volatiles (ca. 150 ng each compound) separated with an HP-INNOWax 30 m x 0.53 mm capillary GC column. All Coleoptera emerged from cut logs of Monterey pine, *Pinus radiata* D. Don. All EAD responses in panel (**B**) were recorded from females with the exception of *L. tuberculatus*, which was a male.

produce or respond (behaviorally) to these EAD-active compounds. Antennal responses of *P. sericeus*, were similar to those of *D. valens*, i.e., they indicated a genera low level "awareness" of semiochemicals produced by *Ips* spp., but nothing can be inferred about the potential for pheromone communication in this species. *Gnathotrichus retusus* is the only species (in the current study) whose pheromone system has been identified, with (*S*)-sulcatol

(not included in our mixtures) as the sole pheromone component (Borden *et al.* 1980). Antennae of this species responded to most of the *Ips* spp. pheromone compounds that we tested (except 2-methyl-3-buten-2-ol and *E*-myrcenol), and the response pattern was similar to that of *I. p. maritimus*, but was weaker in intensity (Fig. 1A). The behavioral activity of these EAD-active compounds in *G. retusus* remains to be explored, except that EAD-active GLVs (C_6-alcohols) interrupted attraction to pheromone-baited traps (Deglow & Borden 1998). (–)-Verbenone has been shown to inhibit the attraction to semiochemicals in over 10 species of bark beetles (Borden 1997). This compound elicited similarly weak but significant EAD-responses from all six of the scolytid species that we tested. Hypothetically, this might indicate a potential behaviorally inhibitory effect on these species. Our data showed more disparities than similarities in EAD-responses to the common pheromone components among the six scolytid species, thus mainly supporting our first hypothesis (H_1).

The antennal responses of *H. tenuis*, *I. p. maritimus,* and *P. sericeus* to 2-methyl-3-buten-2-ol are interesting in the context of the high emission rates of this hemiterpenoid from the foliage of western pines (Harley *et al.* 1998; reviewed in Seybold *et al.* 2006). *Pinus radiata* had an intermediate emission rate relative to nine other species in the survey. The electrophysiological responses of the three scolytids may reflect a potential kairomonal response to the host. If this is the case, then it is interesting that antennae of neither *G. retusus* nor *I. mexicanus* nor the two predators were sensitive to the compound.

Given the results of previous studies of the chemical ecology of *E. sphegeus* in other forest ecosystems, it is not surprising that the antennae of this predator were responsive to some of the *Ips* spp. pheromone components. Attractive kairomonal flight responses have been demonstrated for *E. sphegeus* to ipsenol (Furniss & Livingston 1979) and ipsdienol (Miller & Borden 1990; Seybold *et al.* 1992), whereas the flight response to a generic *Ips* spp./*Dendroctonus* spp. pheromone blend has been interrupted by verbenone (Lindgren & Miller 2002). The flight behavior of *L. tuberculatus* has not been reported, but a related species, *L. subcostulatus* Kraus, responded in a lodgepole pine, *Pinus contorta latifolia*, forest to a similar generic bark beetle attractant, and that response was also interrupted by verbenone (Lindgren & Miller 2002).

Host monoterpenes, major volatile components of many conifer trees (especially *Pinus* spp.), play an important role in host selection of some conifer bark beetles as either attractive kairomones or aggregation pheromone co-attractants (Erbilgin & Raffa 2000; Byers 2004; Seybold *et al*. 2006). Examples of aggregation of bark beetles in response to a kairomone are the mixture of three EAD-active and behaviorally attractive monoterpenes: α-pinene, Δ^3-carene, and terpinolene in *Tomicus piniperda* L. (Byers *et al.* 1985; Schlyter *et al.* 2000) and α- and β-pinene, and Δ^3-carene in *D. valens* (Hobson *et al*. 1993; Erbilgin *et al*. 2007). Many aggressive bark beetles that regularly attack and kill living trees have been shown nearly always to possess an aggregation pheromone, usually of two or more components, but are weakly, if at all, attracted by host volatiles alone (Byers 2004). At the 300 ng level, no EAD-activity was recorded for host monoterpenes by antennae of *G. retusus*, *H. tenuis,* or *I. mexicanus*, whereas only weak (but repeatable) antennal responses were found to α- and β-pinene, and terpinolene by *I. p. maritimus* (Fig. 4A). Surprisingly, the reported antennally (White & Hobson 1993) and behaviorally (Hobson *et al*. 1993) active monoterpenes, α- and β-pinene, and Δ^3-carene were only weakly EAD-active in female *D. valens* in our study; whereas β-myrcene, α-terpinene, (–)-limonene, and terpinolene elicited very strong antennal responses (Fig. 4B). The latter result may have practical importance in the behavioral functionality of these monoterpenes in *D. valens.* Hobson and colleagues studied *D. valens* that originated from a mixed conifer forest ecosystem dominated by ponderosa pine, *Pinus ponderosa* Laws., and this population of the insect may vary from the population in *P. radiata* that we studied here. Although monoterpenes (alone or as co-attractants with scolytid pheromones) have been reported to attract a variety of bark beetle predators (reviewed in Seybold *et al.* 2006), we recorded no antennal responses to this class of compounds by the two predators in our study (Fig. 4B).

The general paucity of EAD-responses to host monoterpenes in most of the species in our study might be indicative of an absence of monoterpene-specific antennal olfactory receptor neurons, extremely high EAD-response thresholds for the monoterpenes, and/or the necessity for simultaneous presentation of some combination of the monoterpenes to elicit a response. α- and β-Pinene are the two major volatile components from sapwood (McDonald *et al*. 1999) and needles (Mateus *et al*. 1997) of *P. radiata.* The EAD-weakly active monoterpenes: α- and β-pinene and terpinolene, on *I. p. maritimus,* might indicate a potential kairomone or aggregation pheromone co-attractant; however, an opposite effect might also be possible. Zhang *et al.* (2007) showed that a mixture of three EAD-active host monoterpenes, α- and β-pinene, and *p*-cymene, was unattractive, but interrupted the response of *Ips subelongatus* Motsch. to its pheromone. Our EAD data do not seem to support our second hypothesis (H_2) that there are no differences in EAD response patterns to common host monoterpenes among the subset of species that we evaluated from the *P. radiata* subcortical insect community; both *D. valens* and *I. p. maritimus* had antennal responses, whereas the rest of the species that we tested did not.

Electrophysiological and behavioral studies have indicated that conifer bark beetles are not only able to recognize, but can also avoid nonhost angiosperm habitats or trees by using olfaction (reviewed in Zhang & Schlyter 2004). Antennal responses to nonhost leaf and bark volatiles have been found by using the coupled GC-EAD technique in over 20 species of bark beetles (Huber *et al.* 2000; Zhang & Schlyter 2004; Shepherd *et al.* 2007).

These antennally active nonhost volatiles (NHVs) such as green leaf volatiles (GLVs) and angiosperm bark volatiles, individually or in various combinations disrupt attractive responses to the pheromone/kairomone systems of many conifer-inhibiting bark beetles (Zhang & Schlyter 2004). No previous electrophysiological data on the responses to NHVs were available on our target species. In the current study, EAD responses to the NHVs were almost identical among the six bark and ambrosia beetle species tested, with *trans*-conophthorin being the strongest in most of the cases, followed by three green leaf alcohols (C_6-alcohols), and the two C_8-alcohols (Fig. 5A,B). Only the antennae of female *P. sericeus* deviated slightly from this pattern with no response to 1-octen-3-ol and a strong response to linalool. The general EAD response pattern to NHVs that we measured to the scolytids is also similar to that of most bark beetle species (Zhang & Schlyter 2004), which supports our third hypothesis (H_3): that there are no significant differences in EAD response patterns to angiosperm NHVs among the subset of phloem- and xylem-feeding species that we evaluated from *P. radiata.* Surprisingly, EAD-responses by scolytids to NHVs were much stronger than to the host volatiles, and in some cases were similar in magnitude to the responses to the pheromone components. Strong EAD-responses to the common NHVs might indicate that they will play an important role in the host selection process of these conifer bark and ambrosia beetles. Although the responses to NHVs were generally lower from the predators than the herbivores, it appears that *E. sphegeus* and *L. tuberculatus* may also use NHVs as olfactory signals. The relatively strong response of *L. tuberculatus* to *trans*-conophthorin is not surprising given attractive field flight responses of other *Lasconotus* spp. to this semiochemical (Dallara *et al*. 2000, Graves 2008). We did not investigate the enantiospecificity of the response by any of the subcortical taxa to *trans*-conophthorin (Zhang *et al*. 2002), which may provide an additional NHV information channel for this community of subcortical insects.

Our GC-EAD data demonstrate that these eight sympatric subcortical beetles, representing seven different genera and two trophic levels related to *P. radiata*, have a broad spectrum of olfactory receptor neurons on their antennae to detect various olfactory signals (in a complex olfactory landscape) from host and nonhost trees, and con- or heterospecific bark/ambrosia beetles. During the dispersal flight for host selection, there are clear advantages for foraging adult beetles to detect and discriminate amongst olfactory signals from hosts and nonhosts, and between con- and heterospecifics from a distance (Byers 1995; Schlyter & Birgersson 1999; Zhang & Schlyter 2004). Our results provide an electrophysiological baseline at the peripheral level for future efforts that may lead to identification of behaviorally active pheromones, host kairomones, and nonhost repellents from the members of this community.

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