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HOST-LOCATION KAIROMONE FROM *Periplaneta americana* (L.) FOR PARASITOID *Aprostocetus hagenowii* (Ratzeburg)[1]

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Abstract—Chemically mediated host location in the eulophid parasitoid *Aprostocetus hagenowii* (Ratzeburg) was investigated. In Y-tube bioassays 77.6% of female parasitoids responded to a *Periplaneta americana* (L.) ootheca; parasitoids did not respond to air with no volatile stimuli. Frass from adult cockroaches was as attractive as an ootheca. Bioassay of one ootheca equivalent of five lipid fractions (eluted with hexane and 1, 5, 10, and 30% ether in hexane) from silica gel column chromatography indicated that the active component was a hydrocarbon. Further separation and bioassay of oothecal hydrocarbons by $AgNO_3$-impregnated silica gel column chromatography indicated that the biological activity was in one fraction. Gas chromatographic (GC) analysis of this fraction revealed a single peak; this peak was identified by researchers in 1963, 1969, and 1972 as (Z,Z)-6,9-heptacosadiene. Qualitative and quantitative GC analyses of total hydrocarbons from oothecae, frass, and adult females were essentially identical; 6,9-heptacosadiene was the dominant hydrocarbon from each source. The alkadiene was 37 times more abundant in frass than on the ootheca. The volatilization of the alkadiene from oothecae was demonstrated by aeration and trapping on Super Q adsorbent. The current study is the first evidence for biological activ-

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ity of (*Z*,*Z*)-6,9-heptacosadiene, a major hydrocarbon component on adult female American cockroaches, on their oothecae, and in their frass.

Key Words—Biological control, cuticular hydrocarbon, attractant, ootheca, 6,9-heptacosadiene, Blattidae, frass, Insecta, *Periplaneta americana*, *Aprostocetus hagenowii*, kairomone.

INTRODUCTION

Kairomone-mediated host location has been well documented. Eggs (Leonard et al., 1987), pupae (Cardé and Lee, 1989), larvae (Vinson et al., 1975), wing scales (Jones et al., 1973; Shu et al., 1990), frass (Jones et al., 1971; Nemoto et al., 1987a,b; Auger et al., 1989; Fukushima et al., 1989), and mandibular gland secretions (Mudd, 1981; Mudd and Corbet, 1982; Mudd et al., 1984) contain compounds that elicit host-finding and host-acceptance behaviors from parasitoids. *Aprostocetus hagenowii* (Ratzeburg), a eulophid parasitoid of oothecae of cockroaches in the family Blattidae, exhibited typical host-recognition behaviors (drumming, tapping, drilling) on glass beads treated with calcium oxalate from colleterial glands and mucopolysaccharides from salivary glands of *Periplaneta americana* (L.) (Vinson and Piper, 1986). *Comperia merceti* (Compere) used the ootheca-fixing cement from the cockroach *Supella longipalpa* as a host-acceptance kairomone (Van Driesche and Hulbert, 1984).

The biology of *A. hagenowii* has been well documented (Roth and Willis, 1954, 1960; Cameron, 1955; Edmunds, 1955; Narasimham, 1984). Its preferred host is an ootheca of *P. americana*, but it will also parasitize oothecae of *P. australasiae* (F.), *P. fuliginosa* (Serville), *P. japonica* Karny, *P. brunnea* Burmeister, *Blatta orientalis* L., *Eurycotis floridana* (Walker), and *E. biolleyi* Rehn. Upon emergence from the ootheca, parasitoid siblings mate and females can immediately oviposit. Developmental time (i.e., oviposition to adult emergence) is approximately 30 days at 30°C; the sex ratio of the resultant clutch is strongly female-biased (86%). The broad host range, fecundity, and life cycle of *A. hagenowii* make it an excellent candidate for the biological control of Blattidae. In this study, we investigated a host-location kairomone for *A. hagenowii* from *P. americana* oothecae.

METHODS AND MATERIALS

Parasitoid. A. hagenowii was colonized in *P. americana* oothecae in the spring of 1993 from 10–20 adult female parasitoids collected at the Swine Research Unit on the University of Florida campus in Gainesville, Florida. A sample of parasitoids was sent to the U.S. National Museum, Washington, D.C., and identified by Dr. M. E. Schauff.

Oothecae were placed individually into 18-ml snap-cap vials (Thornton Plastics, Salt Lake City, Utah) each containing a single 2- or 3-day-old female parasitoid that had emerged from laboratory- and field-stung, sentinel oothecae placed in treehole microhabitats, a primary cockroach harborage in Florida. The vials were stored in plastic boxes (20 × 32 × 10 cm) and held at 27–30°C, in natural light (i.e., by a window) and humidity. Each day the vials containing newly emerged progeny were placed, with snap-caps intact, into a Plexiglas cage (30 × 30 × 30 cm) (Hagenbuch et al., 1988) and held for 24 hr to ensure sibling mating. After 24 hr the snap cap was removed from each vial, allowing parasitoids to mix. Water and food were provided from water and honey–water soaked cotton balls.

A. hagenowii used in this study were 1-day-old (29 ± 11 hr), unfed females that had emerged from three to five laboratory-stung *P. americana* oothecae. The day preceding bioassay parasitoids were released together into a clean Plexiglas cage (Hagenbuch et al., 1988) different from those cages used for parasitoid colony maintenance. The oothecae from which the parasitoids had emerged were removed from the cage to avoid possible habituation to the kairomone.

Oothecae. P. americana oothecae were collected in ootheca production units according to the method of Hagenbuch et al. (1988). Adult cockroaches were obtained as needed from a sewage treatment plant on the University of Florida campus (see Cornwell, 1968, Figure 115). One hundred to 300 mixed-sex adult cockroaches were placed in each tub and provided water from a chick waterer, seven polyvinylchloride harborages (5 cm ID × 12 cm long), and laboratory rat chow (23% crude protein; Purina, #5001, St. Louis, Missouri). Dead cockroaches were replaced, water and food replenished, and oothecae collected weekly.

Olfactometer. A Y-tube olfactometer similar to the one described by Vander Meer et al. (1988) was used in this study. The insect trap was created by a 24/40 ground glass joint. Breathable-quality compressed air was humidified (approximately 75% relative humidity) by passge over 100–200 ml of saturated NaCl solution before being regulated through two flowmeters (65 mm variable area, stainless steel float, 333 ml/min maximum airflow; Cole Parmer, Niles, Illinois) mounted to the inside back wall of a white, wood box (58 cm high × 74 cm wide × 69 cm deep) with an open front and top. The olfactometer was placed on the center floor; a space (3 cm high × 74 cm wide) between the floor and the bottom of the back wall allowed each flowmeter outlet tube to connect to each lateral arm of the olfactometer. All the airflow tubes were corrugated Teflon FEP tubing (Cole Parmer).

A chamber, constructed from the top half of a 50-ml Nalgene bottle (3 cm ID, 1 cm ID at the mouth), was built to allow the unprovoked, natural migration of parasitoids into the common arm of the olfactometer during a bioassay. Into

the bottom of the chamber was a fitted stainless steel screen (150 mesh; Small Parts Company, Miami, Florida) to prevent parasitoids from escaping and to allow the free flow of air from the common arm. Parasitoids were transferred from the Plexiglas holding cage into the chamber through its mouth, which was then fitted to the end of the common arm.

Ootheca and Frass Bioassays. All bioassays were conducted between 09:00 and 17:00 hr at 28–31°C. A single, clean (i.e., without attached detritus), < 10-day-old ootheca was placed distally in a lateral arm of the olfactometer. Ten parasitoids were then placed into the common arm of the olfactometer, and after 10 min the number that (1) moved into the olfactometer trap containing the ootheca, (2) moved into the olfactometer trap containing air only, and (3) failed to enter either trap was recorded. The procedure was repeated but with the ootheca in the other lateral arm of the olfactometer. Thus, a replicate was the response of 20 parasitoids. Each bioassay was conducted with a different cohort of 10 parasitoids. After each bioassay, the olfactometer was disassembled, rinsed with acetone, and fan-dried for 3–5 min. The bioassay was conducted at airflow rates of 30, 60, 120, and 240 ml/min/arm of the Y tube. Finally, the response of parasitoids to air (i.e., no ootheca in either lateral arm) was investigated at the highest airflow rate of 240 ml/min/olfactometer arm.

The bioassay apparatus was used to test 50, 100, and 200 mg samples (N = 2, 3, and 2 replicates, respectively) of 1- to 5-day-old frass from mixed sex adult and large nymphal *P. americana* for its attractiveness to *A. hagenowii*. All frass bioassays were conducted at the highest airflow rate of 240 ml/min/arm.

Isolation of Kairomone from Oothecae. Thirty < 10-day-old oothecae were sequentially immersed in hexane (99.9%; Baxter, Muskegon, Michigan) for 10, 5, 2, and 2 min to remove cuticular lipids. The washes were combined, reduced in volume under a gentle stream of nitrogen and chromatographed on a silica gel column (5 cm) (70–230 mesh; Sigma, St. Louis, Missouri) in a Pasteur pipet. Fractions were collected by gravity elution with sequential 6-ml aliquots of hexane, followed by 1, 5, 10, and 30% anhydrous ether (99.9%; Aldrich, Madison, Wisconsin) in hexane (Carroll, 1961). Each fraction was dried under nitrogen and immediately (< 1 min) reconstituted in hexane to provide one ootheca equivalent (OE) per 10 μl. For each fraction, 1 OE was pipetted onto a paper wick (3 × 14 mm; Northfork, Tumwater, Washington) and bioassayed at the highest airflow rate (240 ml/min/arm); a paper wick control wetted with 10 μl hexane was placed in the opposite lateral arm. A replicate was the response of 20 parasitoids (i.e., 10 parasitoids bioassayed for 1 OE in each lateral arm). Each replicate was expressed as a percentage of the response to an ootheca on the day fractions were bioassayed (i.e., on the same day, the number of parasitoids responding to a fraction divided by the number responding to an ootheca, typically 7–9 of 10).

Cuticular oothecal lipids were removed as described previously, and total hydrocarbons collected by column elution with hexane (Brill and Bertsch, 1985). Total hydrocarbons were then reduced in volume and chromatographed on a column (3 cm) of 10% $AgNO_3$-impregnated silica gel (200+ mesh; Aldrich). Fractions were collected by gravity elution with hexane followed by 2% and 10% ether in hexane (Carlson and Service, 1980). The resulting fractions were passed through a 1-cm column of silica gel and eluted with 2 ml of hexane to remove residual silver ions. Fractions were dried, reconstituted in hexane, and bioassayed.

Collection of Frass and Adult Female Hydrocarbons. Total hydrocarbons from 1- to 5-day-old frass from adult and large nymphal cockroaches and from adult females of unknown age were collected as described previously (Brill and Bertsch, 1985). Frass pellets (200 mg) were immersed in hexane for 24 hr, ground with a glass rod, and rinsed with additional hexane. Thirty adult females were immersed in hexane to remove cuticular lipids. The lipids were reduced in volume by rotoevaporation to 5 ml, and total hydrocarbons were eluted from a silica gel column (1 × 25 cm) with 80 ml hexane.

GC Analysis of Ootheca-, Frass-, and Female-Derived Hydrocarbons. Total hydrocarbons from oothecae, frass, and adult females were analyzed by GC, as were the oothecal hydrocarbon fractions from $AgNO_3$-impregnated silica gel. Additionally, the quantity of 6,9-heptacosadiene was determined for oothecae and frass ($N = 4$ independent total hydrocarbon fractions measured for each) by coinjection of 0.1 μg *n*-hexacosane standard and 0.1 OE of total hydrocarbon or with 0.1 mg equivalent of total hydrocarbon from frass.

Hydrocarbon samples (in 1 or 2 μl hexane) were analyzed with a Tracor gas chromatograph (model 540) equipped with a cool on-column injector, a flame ionization detector (340°C), and hydrogen carrier gas. The capillary column was nonpolar fused silica (DB-1; 30 m × 0.32 mm ID, 0.25-μm film thickness; J & W Scientific, Folsom, California), and the temperature program was 60°C/2 min, 20°C/min to 240°C, held for 0 min, 5°C/min to 320°C, and held for 30 min. Data were recorded with Turbochrom 3 (PE Nelson, Cupertino, California) software with a PE Nelson 900 Series Interface.

Hydrocarbons in this study were identified by chromatogram comparison with published identification of *P. americana* cuticular hydrocarbons (Baker et al., 1963; Beatty and Gilby, 1969; Jackson, 1972), and retention time comparisons with *n*-alkane standards. Baker et al. (1963) and Jackson (1972) identified the alkanes (*n*-pentacosane, 3-methylpentacosane, *n*-heptacosane, and *n*-nonacosane) by their lack of complexing on $AgNO_3$ TLC, response to hydrogenation, inclusion (or not) in a 5 Å molecular sieve, response to bromination, and comparison of GC data from known *n*-alkane standards. 3-Methylpentacosane was further identified by comparison of its mass spectrum with spectra from other cockroach-derived 3-methyl hydrocarbons (3-methyltricosane and 3 meth-

ylheptacosane). Baker et al. (1963), Beatty and Gilby (1969), and Jackson (1972) each identified 6,9-heptacosadiene by its complexing to $AgNO_3$-impregnated silica gel, response to hydrogenation, NMR, IR, UV, and mass spectroscopy. Location of the double bonds was determined by production of both caproic and stearic acids following oxidative cleavage by ozonolysis and potassium-permanganate.

Collection of Volatile 6,9-Heptacosadiene from Oothecae. Compressed air (7.2 liters/hr) was passed through charcoal gravel (#202292, GC hydrocarbon trap grade; Chromatec, Lake Worth, Florida) packed between two, 2-cm-long plugs of highly activated charcoal (Lewcott, Millbury, Massachusetts) in a glass tube (0.6 cm ID). The cleaned air then passed over 175, 1- to 7-day-old oothecae packed into a glass tube (0.6 cm ID). Volatile 6,9-heptacosadiene was collected on 20–30 mg Super Q adsorbent (Alltech, State College, Pennsylvania) held between glass wool plugs and packed into the constriction of a disposable Pasteur pipet attached to the glass tube housing the oothecae. Before volatile collection, the adsorbent was cleaned by elution with 4–5 ml of hexane and dried under a stream of nitrogen. Volatile collections were conducted at 28–30°C. After volatile collection was complete, the adsorbent was eluted with approximately 0.5 ml hexane, concentrated to 0.1 ml, and analyzed by GC-MS. Mass spectral data from volatile 6,9-heptacosadiene was generated on a Perkin-Elmer gas chromatograph (model 8420) interfaced with a Finnigan ion trap. Data were collected and analyzed by INCOS data collection software driven by an IBM-compatible 80286 computer. The capillary column was nonpolar fused silica (DB-1; 30 m × 0.25 mm ID; J & W Scientific). Samples were injected in splitless mode followed, after 30 sec, by a split-purge flow of helium. The helium carrier gas linear flow rate was 25 cm/sec, and the temperature program was 60°C/2 min, followed by 5°C/min to 320°C, and held for 10 min.

RESULTS

Ootheca and Frass Bioassays. A. hagenowii was attracted to volatiles from *P. americana* oothecae and frass. A significantly greater percentage of parasitoids responded to the ootheca at the highest airflow rate than at any of the lower airflow rates (Figure 1). Additionally, at the highest airflow rate parasitoids did not respond when exposed to only air from both lateral arms of the olfactometer; of 100 parasitoids assayed, only one was trapped. *P. americana* frass was also attractive to *A. hagenowii*; 50, 100, and 200 mg of 1- to 5-day-old frass were 97, 124.4, and 96.4% as attractive as an ootheca.

Bioassay of Oothecal Lipids and Hydrocarbons. A. hagenowii was attracted primarily to the hexane fraction (i.e., hydrocarbons) and moderately to the 1%

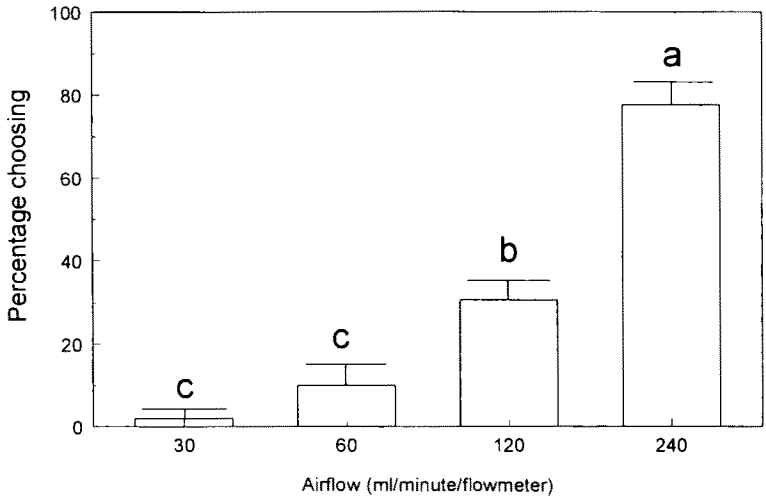

FIG. 1. Ten minute Y-tube bioassay response (mean ± SE, percentage choosing ootheca) of 1-day-old unfed *A. hagenowii* to a < 10-day-old *P. americana* ootheca at each of four airflow rates. $N = 6, 9, 5$, and 5 replicates for the 30, 60, 120, and 240 ml/min/flowmeter airflow rates, respectively. A replicate is the response of 20 insects. Bars topped by different letters are significantly different ($F = 50.4$, $df = 3,45$, $P < 0.0001$) [Tukey's Studentized range test (SAS Institute, 1988)].

ether fraction; fractions eluted with more polar solvent blends were not very attractive (Figure 2A). The hexane and 1% ether fractions were, on average, 77.6 and 19.9%, respectively, as attractive as an ootheca. Bioassay of the hydrocarbon fractions from $AgNO_3$-impregnated silica gel clearly demonstrated that the attractive compound was in the 10% ether fraction (Figure 2B); this fraction was 62.6% as attractive as an ootheca.

GC Analysis of Ootheca-, Frass-, and Female-Derived Hydrocarbons. GC analyses of total hydrocarbon from oothecae, frass, and adult females were qualitatively (Figure 3) and quantitatively (Table 1) very similar; the five dominant peaks constituted 87–96% of the hydrocarbons from each source. The dominant hydrocarbon from each source was 6,9-heptacosadiene (peak 3 in Figure 3). Baker et al. (1963), Beatty and Gilby (1969), and Jackson (1972) had previously found 6,9-heptacosadiene as the major cuticular hydrocarbon on American cockroaches.

GC analysis indicated excellent separation of saturated and unsaturated oothecal hydrocarbons by elution from $AgNO_3$-impregnated silica gel with hex-

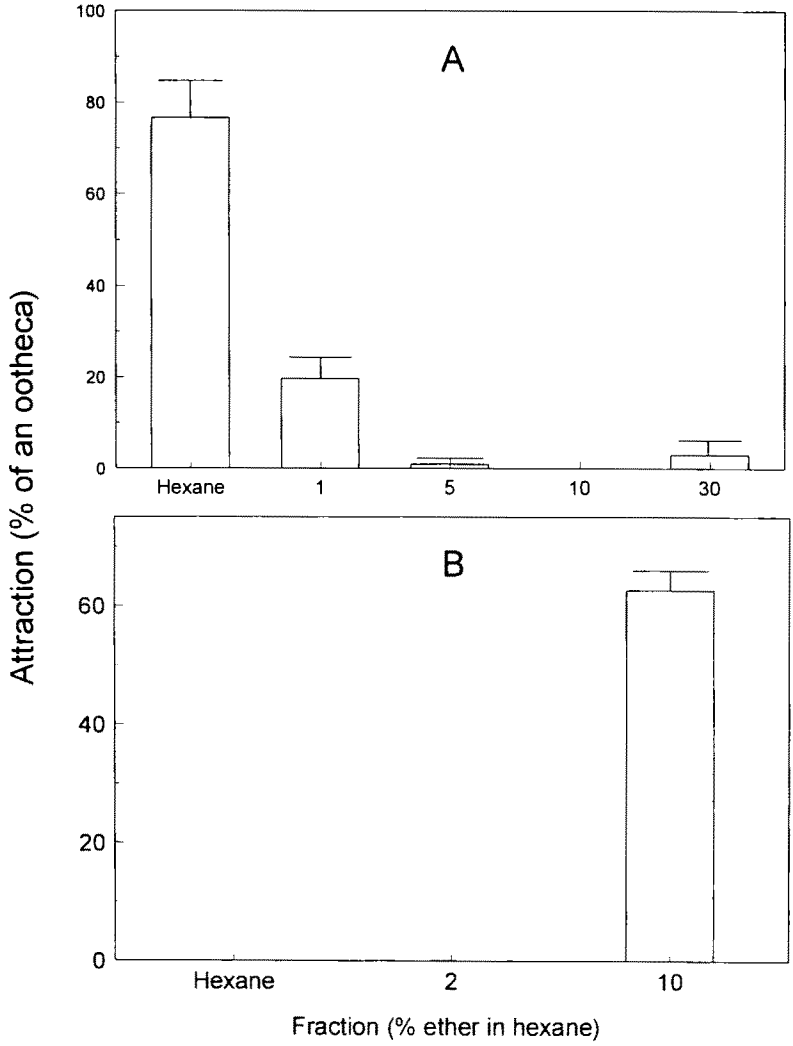

FIG. 2. Attraction (mean percentage of an ootheca ± SE) of 1 OE of (A) cuticular lipids chromatographed on 70–230 mesh silica gel, and (B) cuticular hydrocarbons separated on 10% $AgNO_3$-impregnated silica gel. Attraction is the number of parasitoids responding to a chromatographic fraction divided by the number responding to an ootheca on the same day (i.e., a percentage of the activity of an ootheca). (A) N = 7, 7, 6, 6, and 5 replicates for the hexane, 1, 5, 10, and 30% ether in hexane fractions. (B) N = 6, 6, and 9 replicates for the hexane, 2 and 10% ether in hexane fractions. A replicate is the response of 20 insects.

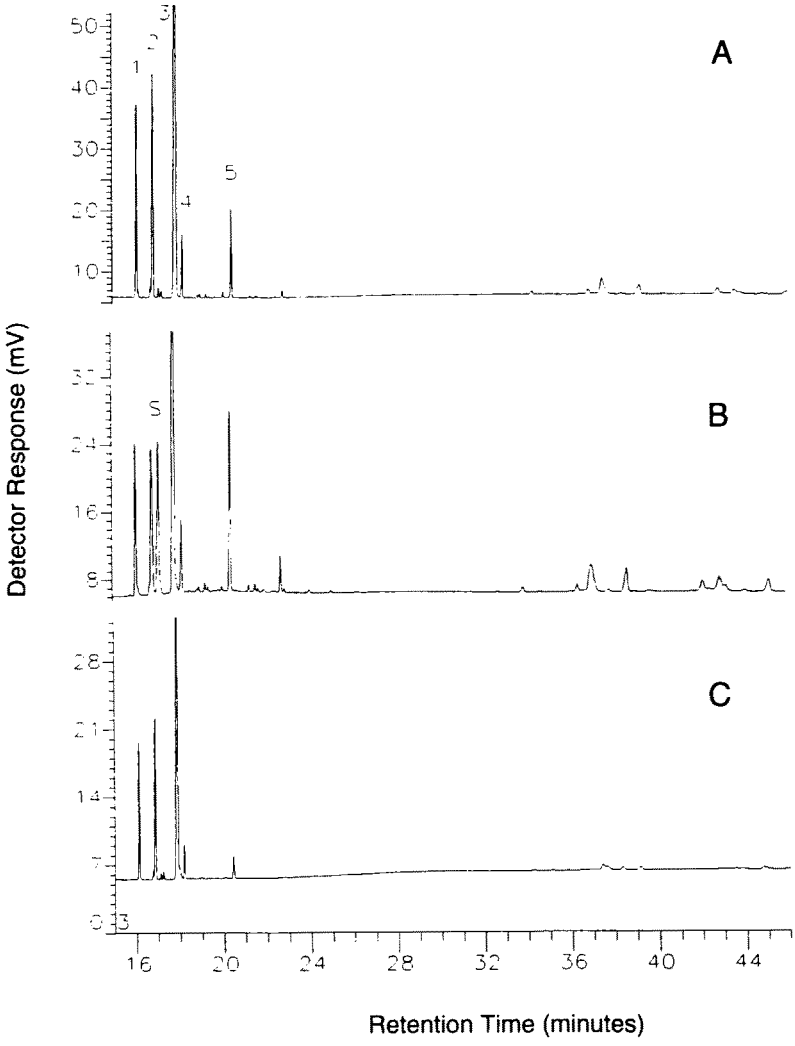

FIG. 3. Gas chromatographic analysis of total hydrocarbons from (A) 1- to 7-day-old *P. americana* oothecae, (B) 1- to 5-day-old frass, and (C) adult females. Hydrocarbon identification after Baker et al. (1963). Peak 1: *n*-pentacosane; 2: 3-methylpentacosane; 3: 6,9-heptacosadiene; 4: *n*-heptacosane; 5: *n*-nonacosane. See Table 1 for corresponding peak area percentages. Peak 3 is off scale to highlight smaller peaks. S is 0.1 μg *n*-hexacosane internal standard. Column: DB-1 (30 m × 0.32 mm ID, 0.25-μm thickness). Temperature program: 60°C/2 min, 20°C/min to 240°C, held for 0 min, 5°C/min to 320°C, and held for 30 min.

TABLE 1. PEAK AREA PERCENTAGE OF HYDROCARBONS FROM *P. americana* OOTHECAE, FRASS, AND ADULT FEMALE

Hydrocarbon source	*N*	% Peak area (mean ± SE)[a]					% of total
		1	2	3	4	5	
Ootheca	5	12.4 ± 0.83	14.7 ± 0.61	60.0 ± 1.04	3.4 ± 0.16	5.5 ± 0.23	96.0 ± 1.71
Frass	4	11.5 ± 1.30	12.9 ± 0.58	45.9 ± 1.10	4.8 ± 0.45	11.9 ± 2.33	87.0 ± 3.45
Female	4	12.9 ± 0.23	15.2 ± 0.18	62.7 ± 0.91	2.9 ± 0.14	3.0 ± 0.06	96.7 ± 0.59

[a] 1 = *n*-pentacosane, 2 = 3-methylpentacosane, 3 = 6,9-heptacosadiene, 4 = *n*-heptacosane, 5 = *n*-nonacosane. Identifications after Baker et al. (1963), Beatty and Gilby (1969), and Jackson (1972).

ane and 2% and 10% ether in hexane (Figure 4). Collectively, Figures 2 and 4 provide strong evidence that the hydrocarbon 6,9-heptacosadiene is the compound responsible for the attraction of *A. hagenowii* to *P. americana* oothecae. This compound was the only one detected in fraction 3 (Figure 4D). There was also a trace amount (2% of total) of diene present in the 2% ether fraction (Figure 4C). The hexane fraction contained only saturated hydrocarbons (Figure 4B).

On a per milligram fresh weight basis, frass contained approximately 37.1 times the amount of 6,9-heptacosadiene as oothecae (surface only). An ootheca extract contained approximately 2.09 ± 0.30 μg 6,9-heptacosadiene, or 0.024 ± 0.003 μg/mg fresh weight (based on an oothecal weight of 87.9 ± 1.29 mg, $N = 9$) and frass contained 0.881 ± 0.10 μg of the alkadiene per milligram fresh weight.

Volatiles. 6,9-Heptacosadiene that volatilized from oothecae was trapped on Super Q (peak 3, Figure 5B). Confirmation of its identity as 6,9-heptacosadiene was determined by several methods. First, via coinjection with alkane standards (*n*-hexacosane and *n*-heptacosane), its Kovats retention index (KI) on a DB-1 column was 2666 (two independent volatile collections); the KI of 6,9-heptacosadiene from *P. americana* oothecae (i.e., Figure 4D) was 2673. In addition, coinjection of oothecal-derived 6,9-heptacosadiene and the volatiles collected from oothecae resulted in elution of a single peak at the retention time of the alkadiene. Finally, the mass spectrum from 6,9-heptacosadiene trapped on Super Q adsorbent was very similar to the spectrum of 6,9-heptacosadiene derived and purified by solvent washing of oothecae; a prominent molecular ion (M^+) of 376 was present in each sample at approximately equal abundance.

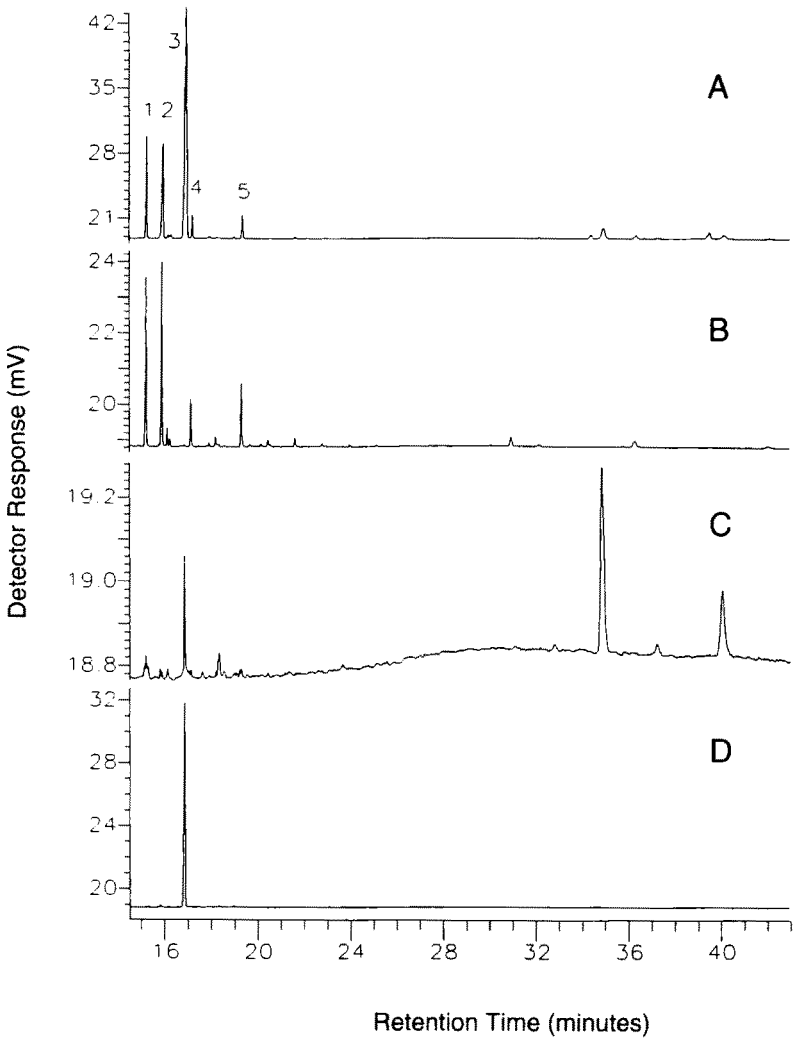

FIG. 4. Gas chromatographic analysis of (A) total hydrocarbons from 1- to 7-day-old *P. americana* oothecae, and oothecal hydrocarbons, which were separated on 10% $AgNO_3$-impregnated silica gel by elution with (B) hexane, (C) 2% ether in hexane, and (D) 10% ether in hexane, with 0.2 OE injected for the hexane, 2%, and 10% ether fractions. Hydrocarbon identification after Baker et al. (1963). Peak 1: *n*-pentacosane; 2: 3-methylpentacosane; 3: 6,9-heptacosadiene; 4: *n*-heptacosane; 5: *n*-nonacosane. The column and conditions are the same as those in Figure 3.

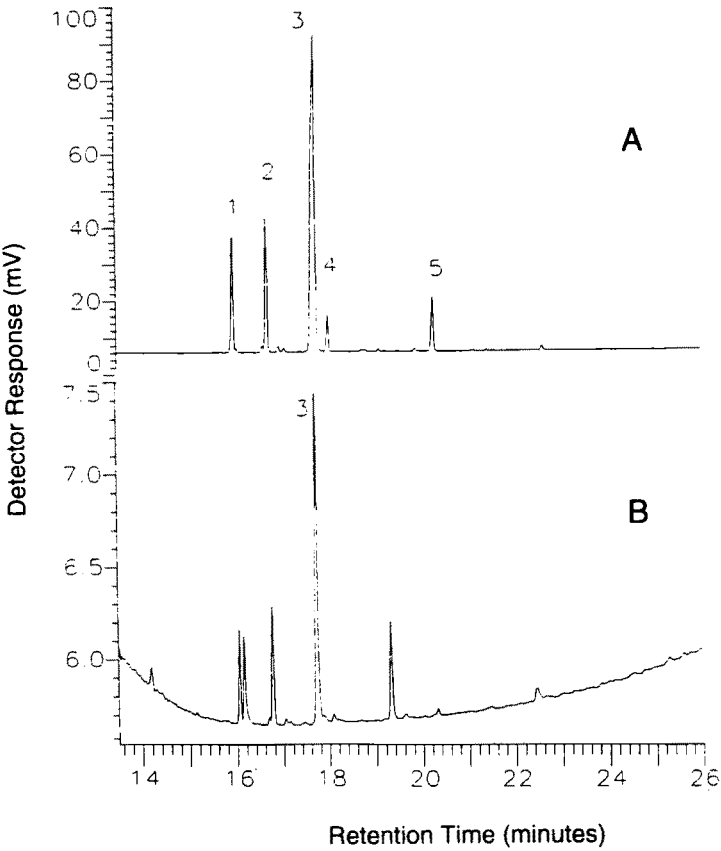

FIG. 5. Gas chromatographic analysis of (A) hydrocarbons from 1- to 7-day-old *P. americana* oothecae, and (B) partial volatiles from < 10-day-old oothecae. Hydrocarbon identification after Baker et al. (1963). Peak 1: *n*-pentacosane; 2: 3-methylpentacosane; 3: 6,9-heptacosadiene; 4: *n*-heptacosane; 5: *n*-nonacosane. The column and conditions are the same as those in Figure 3.

DISCUSSION

The hydrocarbon 6,9-heptacosadiene is used as a short-range kairomone by *A. hagenowii* for locating *P. americana* oothecae. 6,9-Heptacosadiene was identified as a cuticular component in *P. americana* adults (Baker et al., 1963) and is the most abundant lipid constituent of cuticle. Hydrocarbons account for the majority (75–95%) of cuticular lipids in *P. americana* (Gilby and Cox,

1963; Jackson, 1972), and 6,9-heptacosadiene makes up approximately 65–70% of its total hydrocarbon composition. In the present study, total hydrocarbon composition from oothecae, frass, and adult females were qualitatively and quantitatively similar, and paralleled earlier analyses of the hydrocarbon composition of adult *P. americana* (Baker et al., 1963; Beatty and Gilby, 1969; Jackson, 1972).

The current study is the first evidence of biological activity of 6,9-heptacosadiene. Alkadienes with behavioral activity have been found in other insects. For example, 7,11-heptacosadiene, found in the cuticular wax of female *Drosophila melanogaster* (Antony and Jallon, 1982; Antony et al., 1985), has activity in mating bioassays, and 6,9-nonadecadiene is a component of the sex pheromone of several geometrid moth species (Szocs et al., 1984; McDonough et al., 1986). Bartelt et al. (1982) identified a series of 9,19-alkadienes (C_{28}–C_{39}) as the sex pheromone blend of a sawfly, *Pikonema alaskensis*.

The identification of a kairomone in the frass and cuticle of a host was first documented by Jones et al. (1971), who identified 13-methylhentriacontane in the frass, larvae, saliva, and hemolymph of *Heliothis zea* as a host-seeking cue for its parasitoid *Microplitis croceipes*. *P. americana*-infested harborages often contain many kairomone-laden materials such as frass, oothecae, exuviae, and dead cockroaches. The kairomone in these materials may help to maintain a high density of parasitoids in the cockroach breeding area. Lewis et al. (1972) significantly enhanced the parasitism of Lepidoptera eggs by *Trichogramma evanescens* in Petri dishes, greenhouses, and cotton fields by prerelease application of a hexane-soluble host-location kairomone, later identified as *n*-tricosane, from moth scales. Eggs placed in locations treated with the extract suffered higher parasitism than control eggs (i.e., no kairomone). In a later study, Lewis et al. (1975) discovered that broadcast application of *n*-tricosane resulted in increased parasitism of sentinel and wild Lepidoptera eggs by wild and released *Trichogramma* spp. Unlike the inundative release of sex pheromone for mating disruption, large-scale application of a host-seeking stimulant did not confuse the parasitoids; it stimulated host-searching, resulting in increased parasitism.

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