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Cuticular Hydrocarbons of the Ectoparasitic Wasp *Cephalonomia hyalinipennis* (Hymenoptera: Bethylidae) and Its Alternative Host, the Stored Product Pest *Caulophilus oryzae* (Coleoptera: Curculionidae)

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Cuticular hydrocarbons of an ectoparasitic wasp attacking two beetle hosts have been identified and examined for the influence of age, gender, mating status, and host on hydrocarbon composition. The 37 wasp hydrocarbons identified consisted of a series of n-alkanes (C_{16} to C_{33}), 3-, 5-, 9-, 10-, 11-, and 12-methyl alkanes and a series of Z-7 and Z-9 monoenes ($C_{23:1}$ to $C_{27:1}$). One $C_{25:2}$ diene was found. No effects of hydrocarbon composition as a function of age, gender, or mating status were found for the wasps. Wasps reared on *Hypothenemus hampei,* however, had 12/37 significant abundance differences to those reared on *Caulophilus oryzae*, although all but one of these differences were for components in less than 2% relative abundance. The $C_{25:2}$ diene from wasps reared on *H. hampei* was present in about 10% whereas from wasps reared on *C. oryzae* it was present in about 2%. The hydrocarbons of one host for this wasp, the coffee berry borer (Coleoptera: Scolytidae), have been previously reported [Howard and Infante, Ann. Entomol. Soc. Am. *89*:700–709 (1996)]. The hydrocarbons of the alternative host, *C. oryzae* (Coleoptera: Curculionidae) consists of n-alkanes (C_{17} to C_{31}), 3-, 4-, 5-, 7-, 9-, 11-, 12-, 13-, 14-, and 15-methyl alkanes, and a series of dimethyl alkanes of the series 3, 17-; 5, 11-; 5, 17-; 7, 11-; 7, 13-; 13, 17-; and 15, 19-. No unsaturated hydrocarbons were found. No significant differences in hydrocarbon composition were found between male and female *C. oryzae*. Hydrocarbon patterns of four species of *Cephalonomia* are compared and shown to be species-specific. The data are discussed in terms of ecological and physiological parameters. Arch. Insect Biochem. Physiol. 50:75–84, 2002. Published 2002 Wiley-Liss, Inc.[†]

KEYWORDS: semiochemical; chemical ecology; gender recognition; chemotaxonomy; *Cephalonomia hyalinipennis; Caulophilus oryzae; Hypothenemus hampei*

INTRODUCTION

The primitive aculeate family Bethylidae primarily parasitizes small, cryptic larvae of Coleoptera and Lepidoptera (Evans, 1964). These wasps subdue their hosts by multiple stinging and lay one to several eggs externally. The resulting bethylid larvae develop as ectoparasitoids, dropping off the exhausted remains of their host to pupate gregariously in silk cocoons nearby. Males normally emerge before females and may inseminate their sisters or even their mothers in some cases (Evans, 1964).

Several bethylids are commonly associated with the stored commodity environment, including various *Cephalonomia* spp. These parasites are nominally host-specific and can be important biocontrol agents (Flinn and Hagstrum, 1995; Flinn et al.,

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1996; Schöller and Flinn, 2000). Although basic life history data have been gathered on some of these parasites (*C. waterstoni* Gahan: Rilett, 1949; Finlayson, 1950; Howard and Flinn, 1990; *C. stephanoderis* Betrem: Barrera et al., 1989; Abraham et al., 1990; Lauzière et al., 2001; *C. tarsalis* (Ashmead): Powell, 1938; *C. hyalinipennis* Ashmead: Pérez-Lachaud 1998; Pérez -Lachaud and Hardy, 1999), less is known of the biochemistry or chemical ecology of them. Howard (1992) has reported on the cuticular hydrocarbon composition of *C. waterstoni* and its host, *Cryptolestes ferrugineus* (Stephens) and of *C. tarsalis* (Howard, 1998) and its host, the saw-toothed grain beetle (Howard et al., 1995). Howard and Infante (1996) described the cuticular hydrocarbon composition of *C. stephanoderis* and its host, the scolytid beetle *Hypothenemus hampei* (Ferrari) (coffee berry borer).

Cephalonomia hyalinipennis has also been reported to be a native parasitoid of the coffee berry borer in Chiapas, Mexico (Pérez-Lachaud, 1998). It is known to attack a broad spectrum of hosts within the Coleoptera (Gordh and Móczár, 1990; Pérez -Lachaud and Hardy, 1999) and has recently been found to accept facetious alternative hosts, including the stored product weevil *Caulophilus oryzae* (Gyllenhal) (Pérez-Lachaud and Hardy, 2001).

Since most *Cephalonomia* spp. associated with stored products are nominally host-specific, this broader host range of *C. hyalinipennis* offered us the opportunity to comparatively examine potential host-tracking patterns of cuticular hydrocarbons. In this paper, the cuticular hydrocarbon composition of adult *C. hyalinipennis* and its alternative host *C. oryzae* is described and these compositions are compared to other *Cephalonomia* spp. and to the known hydrocarbons of *H. hampei* (Howard and Infante, 1996), another potential host of *C. hyalinipennis*.

MATERIALS AND METHODS

Biological Material

Cephalonomia hyalinipennis were from an experimental rearing unit established in March 1997, initiated with 15 females collected from parasitized coffee berry borers infesting coffee grains (*Coffea canephora* Pierre ex Froehner) (Pérez-Lachaud, 1998). More wasps were collected later and incorporated into the colony stock. The colony was maintained on *H. hampei* hosts for 2 years and then a new colony was initiated using the factitious alternative host *Caulophilus oryzae* (Pérez-Lachaud and Hardy, 2001). This colony has been reared using larvae and pupae of *C. oryzae* for more than 2 years by G.P-L. *Caulophilus oryzae* were from a colony initiated from commercial corn purchased at Mexico City (Pérez-Lachaud and Hardy, 2001). This colony has been maintained on corn for three years. *Hypothenemus hampei* larvae and pupae were from the rearing stock of El Colegio de la Frontera Sur (ECOSUR), at Tapachula, Chiapas, Mexico.

Sample Preparation

Adult *C. hyalinipennis* males and females reared on *C. oryzae*, were collected at emergence and placed in pairs in experimental chambers (two excavated slides put together) until mating occurred. Individuals were then separated and placed in pairs of same sex or individually in a glass vial stoppered with gauze. Virgin females were obtained by removing males before female emergence (mixed broods are usually produced: one male, several females; Pérez-Lachaud and Hardy, 1999). All individuals were provided with diluted honey until extractions were performed. Two individuals per replicate (same age and condition) were extracted. Three replicates of each of the following sets were prepared: (1) 2–3-day-old virgin, honey fed females, (2) 2–3-day-old mated, honey-fed females, (3) 10-day-old mated host fed, ovipositing females, (4) 2–4-day-old mated, honey-fed males, and (5) 2–3-day-old virgin, honey-fed males (2 replications of 5 individuals). Ovipositing females were obtained by providing newly emerged, mated females with 2 *C. oryzae* prepupae or pupae. In addition, three replicates, of five individuals each, of 3-day-old, mated *C. hyalinipennis* males and females reared on *H. hampei*, were also extracted for comparison.

Since *C. oryzae* males and females cannot be

distinguished by size or external morphology, individuals were first extracted in hexane and their sex verified a posteriori by dissection. The age and mating status of extracted individuals were unknown. Three replicates each of one female, one male, and of two late instar larvae were obtained. The initial sample of two larvae of *C. oryzae* yielded almost no hydrocarbons (see Results), so another pooled sample of 9 late-instar larvae was obtained at a later date. A sample of hosts and parasitoids were preserved in 70% ethanol as voucher specimens and were deposited in the entomological collection of El Colegio de la Frontera Sur (Tapachula, Chiapas), at the USDA-ARS, GMPRC, and in the authors' collections.

Extractions

Insects were placed individually or in groups in 5-ml glass vials containing 0.5 ml HPLC-grade n-hexane (Merck, West Germany), and slightly shaken for 1 min. The hexane was transferred by Pasteur pipette to clean 5-ml vials with Teflon®-lined caps and the procedure repeated two more times. The combined portions from each replicate were evaporated at room temperature to almost dryness. Samples were shipped by mail to the Grain Marketing Production Research Center, Manhattan, Kansas, and reconstituted with pesticide-grade hexane prior to analysis by gas chromatography-mass spectrometry.

Hydrocarbon Analysis

Each sample was concentrated under a stream of N_2, and hydrocarbons were isolated by chromatography on a 3-cm "mini-column" of Biosil A (Bio-Rad Laboratories, Richmond, CA) as described earlier (Howard et al., 1978). Electron impact mass spectral analyses were conducted using a Hewlett-Packard 5790A gas chromatograph (GC) (Hewlett-Packard, Inc., San Fernando, CA) containing a DB-5 bonded phase capillary column (20 m long, 0.25 mm inside diameter) (J and W Scientific, Folsom, CA) connected to a Hewlett-Packard 5970 mass selective detector (MSD) and a Hewlett-Packard 9133 data system. Ultrapure helium was the carrier gas, with a column head pressure of 0.75 kg/cm^2. Mass spectra were obtained at 70 eV. Analyses were done using temperature programming, with an initial temperature of 80°C, a final temperature of 320°C, a program rate of 5°C/min, and a 20-min final hold period. The splitless injector was set at 275°C, and the GC/MSD interface was set at 280°C. Retention times of each hydrocarbon component and equivalent chain length values (ECL) were obtained by comparison with known n-alkane standards (Howard et al., 1978). Individual components in the total ion scanning mode were identified from their characteristic EI-MS fragmentation patterns (Jackson and Blomquist, 1976; Nelson, 1978). Quantitative analyses were conducted using the total ion scanning mode and statistical analyses were made using Statgraphics Plus for Windows (Statgraphics, 1998). Means were calculated as proportions and are expressed as percentages in the tables. For ANOVA, proportions were transformed by the arcsine square root transformation before analysis. Separation of means on the transformed data was conducted using the Sequential Bonferroni test (Rice, 1989).

Double-bond locations in alkenes were obtained by preparing dithiomethyl ethers and examining their electron impact mass spectra (EI-MS) (Francis and Veland, 1981). Stereochemistry of the parent alkene was established from comparison of retention times of dithiomethyl ethers of insect-derived olefins to dithiomethyl ethers of known olefin stereoisomers. A DB-5 bonded phase capillary column (20 m long, 0.25 mm inside diameter) using chromatographic conditions identical to those described above was used.

RESULTS

The cuticular hydrocarbons of *C. hyalinipennis* are relatively simple, consisting of a series of n-alkanes (C_{16}–C_{33}), 3-, 5-, 9-, 10-, 11-, and 12-methyl branched alkanes, Z-7 and Z-9 alkenes ($C_{23:1}$–$C_{27:1}$) and one diene, $C_{25:2}$ present in insufficient abundance to locate where the two double bonds are located (Table 1). Multifactorial ANOVA analyses

TABLE 1. Cuticular Hydrocarbons of *Cephalonomia hyalinipennis**

Compound	ECL	CN	Diagnostic mass spectral ions m/z
C_{16}	16.00	16	226
C_{17}	17.00	17	240
C_{18}	18.00	18	254
C_{19}	19.00	19	268
C_{20}	20.00	20	282
C_{21}	21.00	21	296
3-MeC_{21}	21.71	22	281, 253, 310
C_{22}	22.00	22	310
9- & 11-MeC_{22}	22.31	23	141, 211, 324; 155, 197, 324
3-MeC_{22}	22.70	23	267, 295, 324
Z-9-$C_{23:1}$	22.75	23	322 [173, 243, 416]
Z-7-$C_{23:1}$	22.80	23	322 [145, 271, 416]
C_{23}	23.00	23	324
11- & 12-MeC_{23}	23.32	24	169, 197, 338; 183, 338
Z-9-$C_{24:1}$	23.75	24	336 [173, 257, 430]
Z-7-$C_{24:1}$	23.80	24	336 [145, 229, 430]
C_{24}	24.00	24	338
$C_{25:2}$	24.72	25	348
Z-9-$C_{25:1}$	24.75	25	350 [173, 285, 444]
Z-7-$C_{25:1}$	24.80	25	350 [145, 313, 444]
C_{25}	25.00	25	352
5-MeC_{25}	25.60	26	85, 309, 366
$C_{26:1}$	25.75	26	364
C_{26}	26.00	26	366
10- & 11-MeC_{26}	26.33	27	155, 253, 380; 169, 239, 380
Z-9-$C_{27:1}$	26.75	27	378 [173, 313, 472]
Z-7-$C_{27:1}$	26.80	27	378 [145, 341, 472]
C_{27}	27.00	27	380
C_{28}	28.00	28	394
C_{29}	29.00	29	408
C_{30}	30.00	30	422
C_{31}	31.00	31	436
C_{32}	32.00	32	450
C_{33}	33.00	33	464

*ECL, equivalent chain length; CN, carbon number. Ion fragments in brackets are for dithiomethyl ether derivatives.

TABLE 2. Mean Percent (SD) Cuticular Hydrocarbon Composition of Adult *Cephalonomia hyalinipennis* as a Function of Larval Host*

	Host	
Compound	*C. oryzae* (N = 14)	*H. hampei* (N = 7)
C_{16}	0.3 (0.1) a	Tr b
C_{17}	1.1 (0.6) a	Tr b
C_{18}	1.4 (0.7) a	Tr b
C_{19}	1.1 (0.6) a	Tr b
C_{20}	0.8 (0.4) a	Tr b
C_{21}	1.1 (0.5) a	0.2 (0.2) a
3-MeC_{21}	0.2 (0.1) a	Tr b
C_{22}	1.4 (0.7) a	0.4 (0.4) a
9- & 11-MeC_{22}	0.3 (0.2) a	0.1 (0.1) a
3-MeC_{22}	0.2 (0.1) a	0.3 (0.3) a
Z-9-$C_{23:1}$	3.0 (0.6) a	1.3 (0.3) a
Z-7-$C_{23:1}$	1.2 (2.9) a	0.6 (0.3) a
C_{23}	11.4 (4.2) a	15.6 (2.8) a
11- & 12-MeC_{23}	0.2 (0.1) a	0.1 (0.1) a
Z-9-$C_{24:1}$	1.5 (0.4) a	1.4 (0.6) a
Z-7-$C_{24:1}$	0.2 (0.2) a	0.6 (0.3) a
C_{24}	2.9 (1.3) a	1.4 (1.5) a
$C_{25:2}$	2.3 (0.6) a	10.9 (1.6) b
Z-9-$C_{25:1}$	35.2 (10.4) a	42.5 (8.2) a
Z-7-$C_{25:1}$	3.1 (0.9) a	3.8 (0.8) a
C_{25}	8.0 (1.9) a	10.6 (1.4) a
5-MeC_{25}	0.3 (0.1) a	0.3 (0.3) a
$C_{26:1}$	0.3 (0.1) a	0.1 (0.1) a
C_{26}	3.7 (1.8) a	0.8 (1.1) a
10- & 11-MeC_{26}	0.2 (0.1) a	0.1 (<0.1) b
Z-9-$C_{27:1}$	1.0 (0.8) a	0.6 (0.4) a
Z-7-$C_{27:1}$	0.2 (0.1) a	0.6 (0.4) a
C_{27}	6.0 (1.2) a	3.8 (1.5) a
C_{28}	3.2 (1.5) a	0.6 (0.7) b
C_{29}	3.7 (1.2) a	1.7 (1.6) a
C_{30}	1.8 (1.0) a	0.3 (0.3) b
C_{31}	1.5 (0.8) a	0.6 (0.5) a
C_{32}	0.7 (0.5) a	0.2 (0.2) a
C_{33}	0.4 (0.3) a	0.1 (0.1) b

*Means in the same row followed by different letters are significantly different ($P = 0.05$) using the Sequential Bonferroni test. Trace: means and standard deviations less than 0.1%.

of the effects of age, gender, mating status, and host indicated that larval host was the only factor affecting wasp hydrocarbon composition, so samples were pooled, re-analyzed statistically, and presented as percent compositional data as a function of larval host (Table 2). Twelve hydrocarbons were found to vary as a function of larval host. Of these 12, only the $C_{25:2}$ diene represents a component of more than minor abundance (less than 2%) in the wasps. The $C_{25:2}$ diene on wasps reared on *H. hampei* was present in about 10% whereas on wasps reared on *C. oryzae* it was present in about 2%. The short carbon chain length n-alkanes make up most of the 12 components, with wasps reared on *H. hampei* possessing only trace quantities of them. Hydrocarbon composition by class for *C. hyalinipennis* as a function of larval host is found in Table 3.

The cuticular hydrocarbons of adult and larval *C. oryzae* are somewhat more complex than that of the wasps (Table 4). n-Alkanes from C_{17} to C_{31} are found, as well as 3-, 4-, 5-, 7-, 9-, 11-, 12-, 13-, 14-, and 15-methyl alkanes, and a series of dimethyl alkanes with branching occurring at 3, 17-; 5, 11-; 5, 17-; 7, 11-; 7, 13-; 13, 17-; and 15, 19-. No unsaturated hydrocarbons were found. Only one sample of the beetle larvae was available for analysis, and hydrocarbons were present in low abundance compared to the adult stage. All identified hydrocarbons of the larvae were also found

TABLE 3. Mean Percent Hydrocarbon Composition by Class for *Cephalonomia hyalinipennis* as a Function of Larval Host

	Host	
Hydrocarbon class	*C. oryzae*	*H. hampei*
n-alkanes	50.5	36.3
Methyl alkanes	1.4	0.9
Monoenes	45.7	51.5
Dienes	2.3	10.9

on the adults (Table 4), but several other adult hydrocarbons were not detected on the larvae. The percent compositions of the adult beetles as a function of gender and the single analysis of the larvae are shown in Table 5. No significant differences in composition were found between males and females. The composition by hydrocarbon class for *C. oryzae* and *H. hampei* is presented in Table 6.

DISCUSSION

The cuticular hydrocarbon compositions of four species of *Cephalonomia* are now known: *C. waterstoni* (Howard, 1992), *C. stephanoderis* (Howard and Infante, 1996), *C. tarsalis* (Howard, 1998), and *C. hyalinipennis* (this report). Although there are certainly some similarities among these taxa, there are far more dissimilarities. Adult *C. stephanoderis* have the most complex cuticular hydrocarbon profiles of these four bethylids. The major components of adult *C. stephanoderis* are n-alkanes (C_{21}–C_{33}), monomethyl alkanes (3-, 5-, 7-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, and 17-methyl), and Z-10-monomethyl alkenes, with the methyl branch at C_{11} to C_{16}. Minor components include a series of 3,X-, 4,X-, 5,X-, 6,X-, 7,X-, and 8,X-dimethyl alkanes, with X at C_{14} to C_{19}, and a series of 3,9,13-, and 3,11,15-trimethyl alkanes. Male *C. stephanoderis* are characterized by higher proportions of n-alkanes and lower proportions of the monomethyl alkanes and methyl branched alkenes. Female *C. stephanoderis* are characterized by lower proportions of n-alkanes and higher proportions of the monomethyl alkanes and methyl branched alkenes. *C. stephanoderis* had long been thought to be specific to the coffee berry borer but has recently been found to be naturally associated with another scolytid, *Hypothenemus obscurus* Fabricius, in Colombia (Arcila et al., 1997) and to parasitize the alternative factitious hosts *Caulophilus oryzae* and *Sitophilus* sp. in laboratory bioassays (Pérez-Lachaud and Hardy, 2001). The cuticular hydrocarbon profiles of *C. stephanoderis* adults reared on the alternative hosts have not yet been analyzed.

Adult *C. tarsalis,* whose nominal host is the saw-

TABLE 4. Cuticular Hydrocarbons of Adult *Caulophilus oryzae**

Compound	ECL	CN	Diagnostic EI-MS ions, m/z
C_{17}	17.00	17	240
C_{18}	18.00	18	254
C_{19}	19.00	19	268
C_{20}	20.00	20	282
C_{21}†	21.00	21	296
C_{22}†	22.00	22	310
C_{23}†	23.00	23	324
C_{24}†	24.00	24	338
C_{25}†	25.00	25	352
C_{26}†	26.00	26	366
4-MeC_{26}†	26.68	27	71, 337, 365, 380
C_{27}	27.00	27	380
9-MeC_{27}	27.33	28	140, 281, 379
11-MeC_{27}†	27.33	28	168, 253, 379
13-MeC_{27}†	27.33	28	196, 225, 379
7-MeC_{27}†	27.40	28	112, 309 [379]
4-MeC_{27}	27.62	28	71, 351 [379, 394]
7, 11-DiMeC_{27}	27.67	29	112, 323, 183, 253 [393]
7, 13-DiMeC_{27}	27.67	29	112, 323, 183, 253 [393]
3-MeC_{27}†	27.72	28	365 [379]
C_{28}†	28.00	28	394
11-MeC_{28}	28.33	29	168, 267 [393]
12-MeC_{28}†	28.33	29	182, 253 [393]
13-MeC_{28}	28.33	29	196, 239 [393]
4-MeC_{28}†	28.69	29	71, 351, 393, 408
5, 11-DiMeC_{28}	28.84	30	85, 141, 182, 267 [407]
C_{29}†	29.00	29	408
11-MeC_{29}†	29.29	30	168, 281, 407
13-MeC_{29}†	29.29	30	196, 253, 407
15-MeC_{29}	29.29	30	224, 407
7-MeC_{29}	29.38	30	112, 337 [407]
5-MeC_{29}	29.48	30	85, 365 [407]
13, 17-DiMeC_{29}	29.54	31	196, 267 [421]
7, 13-DiMeC_{29}	29.65	31	112, 351, 211, 252 [421]
5, 17-DiMeC_{29}	29.79	31	85, 379, 196, 267, 421
3, 17-DiMeC_{29}	30.06	31	196, 267, 407 [421]
14-MeC_{30}	30.31	31	211, 253 [421]
unknown	30.56	—	85, 127, 211, 267, 421
C_{31}†	31.00	31	[436]
15-MeC_{31}†	31.33	32	224, 253 [435]
7-MeC_{31}†	31.45	32	112, 365 [435]
13, 17-DiMeC_{31}†	31.58	33	197, 295, 225, 267 [449]
13, 17-DiMeC_{35}	35.42	37	197, 351, 267, 281 [505]
13, 17-DiMeC_{37}	37.47	39	197, 379, 267, 309 [533]
15, 19-DiMeC_{37}	37.47	39	225, 351, 281, 295 [533]
13, 17-DiMeC_{39}	39.47	41	197, 407, 267, 337 [561]
15, 19-DiMeC_{39}	39.47	41	225, 379, 295, 309 [561]

*Hydrocarbons with a dagger were also found in larval *C. oryzae.* ECL, equivalent chain length; CN, carbon number. Ions in brackets were predicted but not observed because of low sample abundance.

TABLE 5. Percent Composition of Cuticular Hydrocarbons of *Caulophilus oryzae**

Compound	Mean (SD) (N = 3) Female	Male	Larvae (N = 1)
C_{17}	0.2 (0.1)	0.5 (0.2)	ND
C_{18}	0.4 (0.1)	0.8 (0.4)	ND
C_{19}	0.3 (0.2)	1.1 (0.4)	ND
C_{20}	0.4 (0.1)	0.7 (0.4)	ND
C_{21}	0.3 (0.1)	0.7 (0.4)	0.6
C_{22}	0.4 (0.2)	0.7 (0.1)	0.6
C_{23}	0.5 (0.1)	1.3 (0.5)	0.9
C_{24}	1.0 (0.2)	1.8 (0.8)	0.3
C_{25}	1.3 (0.4)	2.3 (1.0)	0.9
C_{26}	1.6 (0.1)	2.8 (0.6)	0.9
4-MeC_{26}	12.4 (0.8)	11.4 (2.5)	1.1
C_{27}	10.0 (1.5)	8.7 (2.0)	26.6
9-, 11-, 13-MeC_{27}	1.6 (0.5)	2.3 (0.8)	6.6
7-MeC_{27}	0.7 (0.4)	0.8 (0.3)	ND
4-MeC_{27}	0.5 (0.1)	0.9 (0.3)	ND
7, 11-, 7, 13-DiMeC_{27}	0.9 (0.2)	1.3 (0.8)	ND
3-MeC_{27}	0.6 (0.4)	1.5 (0.7)	2.3
C_{28}	1.7 (0.5)	2.6 (0.8)	5.7
11-, 12-, 13-MeC_{28}	0.5 (0.1)	0.6 (0.1)	Trace 12-MeC_{28}
4-MeC_{28}	14.3 (1.7)	9.2 (2.7)	2.9
5, 11-DiMeC_{28}	0.5 (0.1)	0.7 (0.3)	ND
C_{29}	5.4 (1.5)	4.7 (1.4)	35.8
11-, 13-, 15-MeC_{29}	4.8 (0.9)	3.9 (1.3)	8.6
7-MeC_{29}	0.6 (0.1)	0.5 (0.4)	ND
5-MeC_{29}	1.5 (0.2)	1.4 (0.6)	ND
13, 17-DiMeC_{29}	1.3 (0.3)	1.1 (0.5)	ND
7, 13-DiMeC_{29}	1.0 (0.2)	1.2 (0.4)	ND
5, 17-DiMeC_{29}	21.4 (4.1)	19.0 (4.8)	ND
3, 17-DiMeC_{29}	1.6 (0.1)	1.8 (0.1)	ND
14-MeC_{30}	1.0 (0.1)	0.8 (0.2)	ND
Unknown	0.7 (0.1)	0.6 (0.2)	ND
C_{31}	0.8 (0.2)	1.4 (1.0)	1.4
15-MeC_{31}	1.7 (0.2)	1.4 (0.3)	1.4
7-MeC_{31}	1.3 (0.5)	1.3 (0.4)	0.8
13, 17-DiMeC_{31}	0.7 (0.3)	0.7 (0.3)	2.6
13, 17-DiMeC_{35}	1.2 (0.7)	2.0 (0.3)	ND
13, 17-, 15, 19-DiMeC_{37}	3.4 (1.0)	3.6 (0.8)	ND
13, 17-, 15, 19-DiMeC_{39}	1.2 (0.6)	1.0 (0.1)	ND

*ND, not detected.

TABLE 6. Mean Adult Percent Hydrocarbon Composition by Class for *Caulophilus oryzae* and *Hypothenemus hampei*

Hydrocarbon class	*C. oryzae* Female	*C. oryzae* Male	*H. hampei*[a] Female	*H. hampei*[a] Male
n-alkanes	24.3	30.1	13.0	23.3
Monomethyl alkanes	41.5	36.0	47.3	36.9
Dimethly alkanes	33.2	32.4	23.8	29.3
Trimethyl alkanes	0.0	0.0	15.8	10.5
Unknown	0.7	0.6	—	—

[a]Taken from Howard and Infante (1996).

toothed grain beetle (Coleoptera: Cucujidae), has a much simpler cuticular hydrocarbon composition than does *C. stephanoderis*, being composed of n-alkanes (C_{23}–C_{37}), 5-methyl alkanes (5-MeC_{25} to 5-MeC_{29}), 5,17- and 5,19-dimethyl alkanes (5,X-diMeC_{29} and 5,X-diMeC^{33}) and Z-7-, Z-9-, and Z-11-monoenes ($C_{25:1}$–$C_{37:1}$). The alkanes and alkenes are the predominant components of both sexes (>95%), the 5-methyl alkanes accounting for approximately 5% and the dimethyl alkanes present in only trace amounts. Males differ significantly from females with respect to absolute quantity of hydrocarbon per insect in all hydrocarbon classes and in at least 11 major hydrocarbon components, including the C_{25}, C_{27}, and C_{29} monoenes, two n-alkanes, and one 5-methyl alkane (Howard, 1998).

Adult *C. waterstoni*, whose nominal host is the rusty grain beetle (Coleoptera: Cucujidae), possess n-alkanes (C_{23}–C_{27}), 2-, 3- and 5-methyl alkanes (X-MeC_{23} to X-MeC_{27}), 5,15-, 5,17- and 5,19-dimethyl alkanes (5,X-diMeC_{23} to 5,X-diMeC_{25}), and a series of Z-11-monoenes ($C_{25:1}$–$C_{29:1}$). In addition, the males possess small quantities of Z-7-monoenes ($C_{25:1}$–$C_{27:1}$). The males have monoenes and n-alkanes as their major components (38 and 24%, respectively, of total hydrocarbons), whereas the females have monomethyl alkanes and dimethyl alkanes as their major components (39 and 20%, respectively, of total hydrocarbon) (Howard, 1992).

Unlike the three *Cephalonomia* spp previously studied, *C. hyalinipennis* is associated with a broad range of rather disparate hosts, including the coffee berry borer, *H. hampei* (Peréz-Lachaud and Hardy, 1999) and *C. oryzae* (Peréz-Lachaud and Hardy, 2001). Further, *C. hyalinipennis* may act as a facultative hyperparasitoid of both *C. stephanoderis* and *Prorops nasuta* Waterston, another bethylid also attacking the coffee berry borer (G. Pérez-Lachaud, unpublished data). As described above, the cuticular hydrocarbon profile of *C. hyalinipennis* is the most simple of the four species examined to date, with n-alkanes and n-alkenes in approximately equal proportions comprising over 98% of the mixture. A closely related bethylid, *Laelius utilis* Cockerell, a parasitoid of stored product insects and also not highly host specific, has a cuticular hydrocarbon profile that closely resembles that of *C. hyalinipennis*. Its profile has

approximately equal proportions of n-alkanes and alkenes and, as with *C. hyalinipennis,* shows no gender related differences in hydrocarbon composition (Howard, 1992).

A detailed comparison on a presence/absence basis of the cuticular hydrocarbons of the four *Cephalonomia* spp. examined to date revealed that 136 different hydrocarbons are present among them (Howard, 1998; Howard and Infante, 1996; this report). The list includes 22 n-alkanes, 43 monomethyl alkanes, 23 dimethyl alkanes, four trimethyl alkanes, 42 monoenes, and two dienes. By species, *C. hyalinipennis* has 37 hydrocarbons, *C. waterstoni* has 39 hydrocarbons, *C. tarsalis* has 45 hydrocarbons, and *C. stephanoderis* has 69. In terms of shared components, all four species share only eight compounds, all of them being n-alkanes. Seven compounds are shared by three of the species: four n-alkanes, two 5-methyl alkanes, and one monoene. Fourteen compounds are shared by two of the species, and 107 compounds are found only in a single species. These species are not easy to collect, so that no analyses have been obtained from different populations of each species. It is inappropriate, accordingly, to use multivariant methods to establish relationships among the four species, three of which are found associated with stored product pests (*C. stephanoderis*, the other species, is usually found associated with field populations of the coffee berry borer). It appears, however, that all have species-specific hydrocarbon profiles that could be used for the development of a taxonomic key for identifying species on the basis of their cuticular hydrocarbons when additional populations become available for analysis.

It is becoming increasingly evident that the cuticular hydrocarbon profiles of many insects are not static, but rather respond dynamically to ecological, environmental, ontogenetic, and physiological factors (Howard, 1993; Howard and Akre, 1995; Howard et al., 1995; Lockey, 1988; Schal et al., 1994). We examined whether the cuticular hydrocarbon profile of *C. hyalinipennis* varied as a function of age, gender, mating status, and larval host. We found that age, gender, and mating status did not influence their hydrocarbon compositions, whereas a number of differences were found as a function of larval host. These differences, although highly significantly different ($P < 0.001$), were found for minor components present in less than 2% (Table 2). The one exception is the $C_{25:2}$ diene, which is present in about 10% relative abundance in wasps reared on *H. hampei*, but is present in slightly more than 2% abundance in wasps reared on *C. oryzae*. It is not clear why this fivefold difference occurs, and further analysis will be required to clarify the matter. The bulk of the quantitative difference between parasitoids reared on the two hosts arises from this one compound, however (Table 3). One possibility why at least some of the hydrocarbons of *C. hyalinipennis* differ in relative abundance as a function of larval host is that the wasps depend on a dietary incorporation of some host lipids, although few studies have unequivocally demonstrated direct acquisition and transport of dietary lipids to the cuticle (Howard and Blomquist, 1982, Blomquist et al., 1993).

Unlike *C. hyalinipennis*, distinct life-stage and gender-related differences in hydrocarbon composition were found for the other *Cephalonomia* spp. examined (Howard, 1992, 1998; Howard and Infante, 1996). In addition, for *C. tarsalis*, a distinct association of reproductive and host-feeding status on cuticular hydrocarbon composition was found (Howard, 1998). Further studies will be required with *C. hyalinipennis* to clarify whether they too are affected by these variables or whether there is something peculiar to their biology, possibly related to their characteristic polyphagy, that makes them relatively immune to such factors. Of course, compounds (and other cues) other than cuticular hydrocarbons are well known as insect pheromones, and gender discrimination and sexual stimulation may be achieved by *C. hyalinipennis* using these sorts of stimuli. Only future studies will be able to answer this question.

We were also interested in whether the two alternative hosts of *C. hyalinipennis* would have similar cuticular hydrocarbon profiles and whether the wasp would track these profiles with its own com-

position. Even though the hydrocarbons of both beetle hosts are comprised solely of saturated hydrocarbons, the individual components and class composition of the hydrocarbons differ considerably (Table 6). Interestingly, this difference does not preclude the parasitization of both hosts species, not only by *C. hyalinipennis* but also by *C. stephanoderis*, which was thought to be highly host specific, suggesting that cuticular hydrocarbons may not be the critical cues involved in the host acceptance process by females of these two *Cephalonomia* spp. In a previous study, Lauzière et al. (2001) suggested that essential chemical and physical cues from *H. hampei* hosts (prepupae and pupae) were perceived by *C. stephanoderis* females upon contact, and were likely to be involved in triggering both oogenesis and egg laying. More studies are needed to understand the proximal causes of host acceptance process in species with either a broad range of hosts like *C. hyalinipennis* or in those with narrower host ranges like *C. stephanoderis.*

Furthermore, the wasp hydrocarbons do not closely track either set of host hydrocarbons. Indeed, all four *Cephalonomia* species examined differ substantially from their hosts' cuticular hydrocarbon profiles. The cuticular hydrocarbon profiles of the beetle hosts associated with the four *Cephalonomia* spp. also differ greatly from one another. Clearly, even in the absence of biosynthetic studies, we can unequivocally assert that these wasps are making their own hydrocarbons, and not acquiring them from their hosts to any substantial extent. The hydrocarbons shared by wasps and hosts represent only 35.9% for *C. waterstoni* (14/39), 49.3% for *C. stephanoderis* (34/69), 35.4% for *C. tarsalis,* and 37.8 % for *C. hyalinipennis* (14/37) reared on either *C. oryzae* or *H. hampei* (Howard, 1992; Howard and Infante, 1996; Howard et al., 1995; current work). This may be contrasted to the ratio of hydrocarbons shared by the parasitoid *Kapala sulcifacies* (Cameron) (Hymenoptera: Eucharitidae), an inquiline of the ponerine ant *Ectatomma ruidum* Roger (Hymenoptera: Formicidae). Here the female parasitoids share 40/55 (92.6%) and the male parasitoids share 40/54 (84.3%) of the ant hydrocarbons (Howard et al., 2001). Unlike the situation with the *Cephalonomia* spp., however, the *Kapala* hosts must detect and kill all alien nestmates, and the high degree of correspondence between the *Kapala* hydrocarbon profile and that of their ant hosts is necessary for the survival of the parasitoid until they can exit the ant colony.

Cuticular hydrocarbons are known to function as species, gender, and colony recognition cues, and as pheromones, allomones, and kairomones in a diversity of ecological situations (Howard and Blomquist, 1982; Blomquist et al., 1993; Howard, 1993). Precedence exists in parasitic wasps for cuticular hydrocarbons serving as close-range species-, gender-, and host-recognition cues at the individual level (Howard, 1998; Howard et al., 1998), and recent behavioral data on interspecific interactions between coffee berry borer parasitoids seem to suggest a similar phenomenon. In the bethylids *C. stephanoderis, C. hyalinipennis*, and *P. nasuta*, Pérez-Lachaud et al. (2002) have experimentally shown the existence of aggressive host and brood guarding behavior: direct behavioral contests for hosts occur between adult female parasitoids, the loser being frequently killed. Although the stimuli that release biting and stinging behaviors have not been identified yet, it is likely that females of these three species recognize intruders based on close-range cues because females do not attack each other on first encounter and have been observed to repeatedly touch each other with their antennae before attacks begin. It remains to be seen if these close-range cues are the species-specific cuticular hydrocarbons or possibly other cuticle-borne chemical cues.

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