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(*Z*)-9-Pentacosene – contact sex pheromone of the locust borer, *Megacyllene robiniae*

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Summary. Male locust borers, *Megacyllene robiniae* (Förster), responded to females only after contacting them with their antennae, indicating that mate recognition was mediated by a contact sex pheromone. GC-MS analyses of whole-body extracts of males and females determined that the profiles of compounds in the extracts were qualitatively similar, but differed considerably in the ratios of compounds between sexes. Biological activities of reconstructed blends of the most abundant straight-chain (nC_{23}, nC_{24}, nC_{25}, nC_{26}), methyl-branched (3me-C_{23}, 3me-C_{25}), and unsaturated ($Z9$:C_{23}, $Z9$:C_{25}, $Z9$:C_{27}) compounds in extracts from females were assessed in arena bioassays, assessing four distinct steps in the mating behavior sequence of males (orientation, arrestment, body alignment, mounting and attempting to couple the genitalia). Males were unresponsive to freeze-killed, solvent-washed females treated with blends of straight-chain and methyl-branched alkanes, but responded strongly to females treated with the blend of alkenes. Further trials determined that the complete sequence of mating behaviors, up to and including coupling the genitalia, was elicited by $Z9$:C_{25} alone. $Z9$:C_{25} comprised $16.4 \pm 1.3\%$ of the total hydrocarbons in whole-body hexane extracts of females and was co-dominant with two other hydrocarbons that were not active. In contrast, in solid phase microextraction (SPME) wipe samples from several areas of the cuticle, $Z9$:C_{25} appeared as the single dominant peak, comprising 34.6 – 37.8% of the sampled hydrocarbons. Our data indicate that $Z9$:C_{25} is a contact sex pheromone of *M. robiniae*, being the most abundant hydrocarbon on the surface of the cuticular wax layer of females where it is readily accessible to the antennae of males.

Key words. Cerambycidae – mating behavior – cuticular hydrocarbon – (*Z*)-9-pentacosene – solid phase microextraction

Introduction

The wax layer on the cuticle of insects is comprised of a complex mixture of long-chain fatty acids, alcohols, esters, aldehydes, ketones, and hydrocarbons that protect insects from desiccation (Gibbs 1998). Components of the wax layer also may have a secondary role as contact pheromones (Blomquist *et al.* 1996). There is a growing body of evidence that mate recognition in cerambycid beetles is mediated by such contact sex pheromones (Kim *et al.* 1993; Fukaya *et al.* 1996, 1997, 2000; Wang 1998; Ginzel *et al.* 2003; Ginzel & Hanks 2003). We report here the results of studies on a cuticular sex pheromone of the locust borer, *Megacyllene robiniae* (Förster) (Coleoptera: Cerambycidae). This black and yellow longhorned beetle is native to temperate North America and causes substantial damage to poorly managed and older black locust trees, *Robinia pseudoacacia* L., as well as to naturally regenerating locust trees and those in young plantations (Solomon 1995). *Megacyllene robiniae* is univoltine, with adults emerging in late summer and early fall. Adults aggregate on inflorescences of goldenrod (*Solidago* spp.) where they feed on pollen and mate (Galford 1984). Females then fly to black locust trees on which they deposit eggs singly in bark fissures and around wounds. The young larvae overwinter, then complete their development in the inner bark and wood the following spring and summer, pupating in late summer (Solomon 1995).

The mating behavior of *M. robiniae* has been studied (Ginzel & Hanks 2003). Males orient to females only after contacting them with their antennae, and mate recognition is cued by contact chemoreception. After touching a live or freeze-killed female with his antennae, a male *M. robiniae* aligns his body with that of the female, and attempts to copulate, bending his abdomen to couple with the female's genitalia (Ginzel & Hanks 2003). Males do not display any of these behaviors when presented with solvent-extracted, freeze-killed females, but reapplying the extract to solvent-washed female carcasses restores activity, demonstrating that extractable chemical cues in the cuticular wax layer trigger mate recognition and subsequent mating behaviors (Ginzel & Hanks 2003).

In this article, we identify a female-produced contact sex pheromone for *M. robiniae*. Identification was accomplished using two methods of sampling cuticular components, the traditional whole-body solvent extraction, and solid phase microextraction (SPME). SPME has been used as an alternative to solvent extraction in a number of recent papers on cuticular hydrocarbons of insects (e.g., Turillazzi *et al.* 1998; Peeters *et al.* 1999; Liebig *et al.* 2000; Sledge

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et al. 2000; Roux *et al.* 2002) and reportedly yields samples that are qualitatively and quantitatively similar to those obtained by solvent extraction (Moneti *et al.* 1997; Monnin *et al.* 1998; Bland *et al.* 2001; Tentshert *et al.* 2002).

Materials and methods

Sources of beetles

For our experiments, we collected beetles at Phillips Tract (Champaign County, Illinois), Allerton Park (Piatt County, Illinois), and Kickapoo State Recreation Area (Vermilion County, Illinois). In the laboratory, males and females were housed separately in 0.3 m^3 cages of aluminum window screen, with cages held under ambient laboratory lighting and temperature conditions. Caged beetles were provided fresh goldenrod inflorescences with feeder vials of 10% sucrose solution (glass vial with a cotton dental roll, Patterson Dental Supply, South Edina, MN), and feeders were replaced every 2–3 days. The beetles used in preparation of extracts and in bioassays were vigorous and active in cages.

Preparation of whole-body extracts and identification of cuticular hydrocarbons

Cuticular chemicals were extracted from ten freeze-killed virgin female and male *M. robiniae* by individually immersing each beetle in two 1 ml aliquots of hexane for 2 min each. Aliquots were combined and concentrated to 1 ml under a stream of nitrogen. Extracts were initially analyzed at the University of Illinois at Urbana-Champaign (UIUC) by coupled gas chromatography – mass spectrometry (GC-MS) with electron impact ionization (70 eV) using a Hewlett-Packard 6890 GC interfaced to an HP 5973 mass selective detector (Hewlett-Packard, Sunnyvale, CA), equipped with an HP-5MS (Hewlett-Packard) capillary column (30 m × 0.25 mm × 0.25 µm film thickness) in splitless mode with helium as the carrier gas. After a 1 min hold at 50º C, the oven temperature was ramped to 240ºC at 10º C/min, then 240 to 300ºC at 2º C/min with a 5 min hold at 300ºC. Injector and transfer line temperatures were 280ºC. Quantitative data presented in Table 1 and Figures 1 and 2 were produced by these analyses.

Compounds were identified in the laboratory of JGM with an HP 6890 GC interfaced to an HP 5973 mass selective detector (Hewlett-Packard, Sunnyvale, CA), with a DB-5 (J&W Scientific, Folsom, CA) capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The temperature program was 100ºC for 1 min, 10 ºC/min to 280ºC, and held at 280ºC for 20 min. Injector and transfer line temperatures were 280ºC. Cuticular hydrocarbon components were identified by comparison of mass spectra and retention times with those of standards, or from the parent M^+ ions and the corresponding molecular formulae, retention times relative to straight-chain compounds, and diagnostic mass spectral fragments that unequivocally demonstrate the position of methyl branches, as thoroughly documented by previous researchers (e.g., Nelson 1993; Nelson and Blomquist 1995). The identities of the straight-chain alkanes, the most abundant monoenes, 3-methyltricosane (3-meC_{23}), and 3-methylpentacosane (3-meC_{25}) were confirmed by matching their retention times and mass spectra with those of authentic standards (see below). Double bond locations and geometries of monoenes were determined by epoxidation of extracts (see below) followed by GC-MS analysis of the resulting derivatives. The epoxides gave large diagnostic fragments from cleavage on either side of the epoxide, unequivocally demonstrating the position of the double bond in the parent molecule. GC-MS analysis of the epoxides of *E* and *Z* isomers determined that the *E* isomers eluted before the *Z* isomers on the DB-5 column.

Solid phase microextraction

We sampled cuticular components of female *M. robiniae* with a 100 µm film thickness polydimethylsiloxane SPME fiber (Supelco Inc., Bellefonte, PA). Cuticular chemicals were sampled by gently rubbing the dorsal surface of the elytra with the SPME fiber for 30 sec, and we sampled five beetles of each sex. To assess the consistency in the chemical composition of the wax layer surface across body regions, we sampled ventral surfaces of the elytra, thoracic tergites, and abdominal sternites, for an additional three females. Samples were analyzed at UIUC by GC-MS by desorbing the SPME fiber for 1 min in the injection port (250ºC) using the same temperature settings used in analyzing extracts (see above).

Chemical standards

C_{22}–C_{30} straight-chain hydrocarbons were purchased from Sigma-Aldrich Co. (St. Louis, MO), and Alltech Associates (Deerfield, IL). Other standards were obtained as follows: 3-meC_{23} (Analabs, North Haven, CT), 3-meC_{25} (extracted from American cockroaches, *Periplaneta americana* (L.) as described by Ginzel *et al.* 2003), and (*Z*)-9-tricosene (Lancaster Synthesis, Wyndham, NH).

(*Z*)-9-pentacosene and (*Z*)-9-heptacosene were synthesized as follows: Sodium hexamethyldisilazide (1M in THF, 5 ml, 5 mmol; Aldrich) was added dropwise to a cooled (0ºC) solution of (1-nonyl)triphenylphosphonium bromide (2.35 g, 5 mmol; Lancaster Synthesis) in 20 ml THF. The bright orange solution was stirred 1 hr at 0ºC, then cooled to –78ºC in a dry ice-acetone bath, and a solution of hexadecanal (0.73 g, 3 mmol) in 5 ml THF was added dropwise over 10 min. The mixture was warmed to room temperature overnight, then quenched with water and extracted with hexane. The hexane solution was backwashed with brine, dried over anhydrous Na_2SO_4, then concentrated. The residue was taken up in hexane and purified by vacuum flash chromatography (Coll & Bowden 1986) on silica gel, eluting with hexane. The purified hydrocarbon fraction was concentrated by rotary evaporation, pumped under vacuum to remove traces of solvent, then dissolved in 20 ml warm acetone and recrystallized at –20ºC, yielding *Z*9:C_{25} as a low-melting solid (690 mg; chemical purity > 97%). (*Z*)-9-heptacosene was made in analogous fashion and purity, using octadecanal as the starting material.

Epoxidation of a sample with meta-chloroperbenzoic acid in methylene chloride at room temp for 2 hr, followed by GC-MS analysis determined that the *E*:*Z* ratio of *Z*9:C_{25} was 3:97 (see below). To confirm the *E*:*Z* ratio and elution order of the alkenes, 5 mg of each was treated with 1.5 µl of thiophenol in a sealed vial at 110ºC for 1 hr. The resulting mixture, containing predominantly the thermodynamically more stable *E* isomer, was taken up in pentane, washed thoroughly with 1M NaOH to remove the thiophenol, and epoxidized as described above. On a DB-5 column, the *E* isomers eluted before the *Z* isomers.

Testing the activity of hydrocarbon standards

Hexane solutions of hydrocarbon standards were prepared in concentrations of 1 female equivalent (FE) in 100 µl of hexane which approximated the original extracts of female *M. robiniae* (quantified by comparing peak areas in integrated MS total ion chromatograms with an internal standard). Blends of hydrocarbons were based on chemical class, that is, straight-chain alkanes, monomethyl-branched alkanes, and alkenes (see Results). We tested the bioactivity of blends and individual compounds with the following bioassay (see Ginzel *et al.* 2003):

1) A female was freeze killed (–4ºC for 20 min), allowed to warm to room temperature (~15 min) and presented to a male in a clean glass Petri dish lined with filter paper. An attempt to mate was scored as evidence that recognition cues were intact and that a behavioral response by the female was not necessary for mate recognition.
2) Cuticular components were removed from the dead female by immersing her in two sequential 1-ml aliquots of analytical-grade hexane for 2 min each.
3) The solvent-washed female carcass was air dried for 30 min to allow evaporation of hexane and then presented to the same male. Lack of a response by the male was scored as evidence that chemical recognition cues had been eliminated.

4) To test bioactivity of extracts, fractions of extracts, and individual or blends of hydrocarbon standards, the appropriate solutions in hexane were pipetted onto the body of a solvent-washed female, the solvent was allowed to evaporate, and the treated carcass was again presented to a male beetle. Because males responded to 0.2 female equivalents (FE) of crude extracts of females (Ginzel & Hanks 2003), we applied a similar dose of standards to the solvent-washed female carcass, and as a control, treated a second solvent-washed female carcass with 20 µl of hexane. The 0.2 FE dosage of standards contained 44 µg of the most abundant compound, nC_{25} (see Results), with concentrations of other standards approximating their relative ratios in the crude extract (see Table 1). The two carcasses were then presented to the male simultaneously, on opposite sides of the Petri dish arena, with their positions randomized to control for location effects.

Each treatment was tested with 16 different males, videotaping the behavior of each male in the Petri dish arenas. Males were housed individually in aluminum window screen cages to ensure that they had not mated for 24 hr prior to bioassays, and individual beetles were used in bioassays only once per day. After a male contacts a living female with his antennae, a clear progression of behaviors leads to copulation: 1) the male orients to (turns toward) the female, 2) stops walking (= arrestment), 3) aligns his body with the female, and 4) mounts her and attempts to couple the genitalia. Thus, our assessment of behavioral response was cumulative: males must perform steps 1 through 3 to reach step 4.

A trial was scored as a "response" if the male displayed at least the first behavior (turning toward female), within 5 min after first contacting a treated or control female carcass with his antennae. A trial was scored as "no response" if the male showed none of these behaviors within 5 min of initial antennal contact, but rather continued to walk after contacting either a treatment or a control female with his antennae. The numbers of males responding to treatments versus controls were compared with the χ^2 goodness-of-fit test (Sokal & Rohlf 1995).

Results

Preliminary bioassays

As a prelude to further investigation, we determined that male *M. robiniae* could discriminate between female and male conspecifics by contact chemoreception. When we caged pairs of males with single females in Petri dish arenas (N = 55 trials), every male attempted to mate with the female after first antennal contact, but none of the males showed any of the mating behaviors after contacting another male. Males only briefly antennated other males, then avoided them. Response of male *M. robiniae* to females and other males in the field is consistent with these laboratory studies (MDG, pers. obs.).

Identification of cuticular hydrocarbons

Hexane extracts of female and male *M. robiniae* consisted predominantly of saturated and unsaturated hydrocarbons (Figure 1, Table 1), and there were consistent, sex-specific differences in the hydrocarbon profiles of males and females. Dominant compounds in extracts from females included nC_{23} (Peak 4, Figure 1), $Z9{:}C_{25}$ (Peak 9), and nC_{25} (Peak 11). Extracts of males contained almost all of the same compounds present in females, missing only peaks that were minor in female extracts (peaks 1, 17, 22; Table 1). nC_{25} also was a dominant compound in males, and there were no compounds that were specific to males (Figure 1,

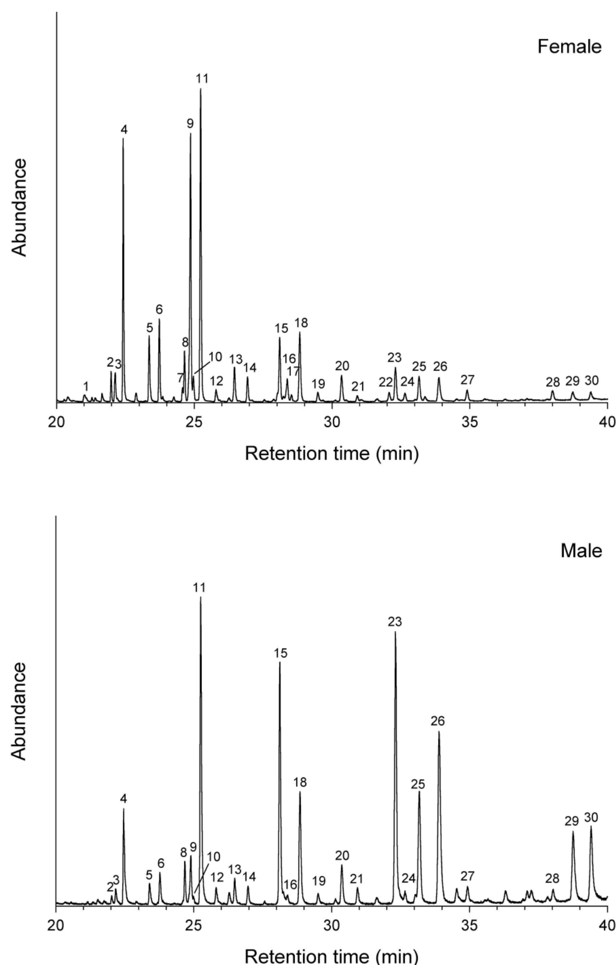

Fig. 1 Representative total ion chromatograms of hexane extracts of an adult female (top) and male (bottom) *Megacyllene robiniae*. Analysis conditions are described in Materials and methods

Table 1). Ratios of compounds varied considerably between the sexes, however, with males having greater amounts of compounds with longer chain lengths, particularly alkanes with a single methyl branch (Figure 1, Table 1).

Solid phase microextraction

Sampling cuticular hydrocarbons from female and male *M. robiniae* with a SPME fiber yielded a markedly different profile of compounds than the whole-body solvent extracts (Figure 2 compared to Figure 1, Table 1). The chromatogram from the SPME wipe sampling of the elytra of females showed a dominant peak, $Z9{:}C_{25}$ (Peak 9, Figure 2), which comprised 37.8 ± 1.3% of the total hydrocarbon content in SPME samples, compared to only 16.3 ± 1.3% in the solvent extract (means significantly different, ANOVA $F_{1,9}$ = 150.0, $P < 0.0001$). SPME sampling of females did not reveal any compounds that were not present in the whole-body extracts; in five instances, compounds present in the whole-body extracts were not detected in the SPME samples (Table 1). In the latter case, these were primarily the longer-chain components. The relative proportion of $Z9{:}C_{25}$ to other

Table 1 Cuticular hydrocarbons of female and male *Megacyllene robiniae*. Peak numbers correspond to those in Figure 1. Percent of total hydrocarbons represent means ± 1 SE for five individuals. "nd" = not detected

			% of total hydrocarbons ± 1 SE				
			Hexane Extracts		SPME Samples		
Peak Number	Retention Time	Hydrocarbon	Female	Male	Female	Male	Diagnostic Ions
1	21.13	nC_{22}	0.32 ± 0.11	nd	nd	nd	310 (M^+)
2	21.99	2-meC_{22}	1.15 ± 0.13	0.12 ± 0.08	1.00 ± 0.30	nd	324 (M^+), 309, 281
3	22.13	Z9:C_{23}*	1.85 ± 0.23	0.66 ± 0.19	3.40 ± 0.43	nd	322 (M^+), 83, 97, 111
4	22.42	nC_{23}	11.9 ± 0.68	4.56 ± 1.0	6.36 ± 0.34	1.17 ± 0.74	324 (M^+)
5	23.36	3-meC_{23}	5.31 ± 1.0	1.66 ± 0.50	5.81 ± 0.94	nd	338 (M^+), 309
6	23.73	nC_{24}	5.58 ± 0.33	2.03 ± 0.41	2.24 ± 0.27	0.26 ± 0.17	338 (M^+)
7	24.57	4-meC_{24}	0.48 ± 0.25	0.39 ± 0.26	1.39 ± 0.10	nd	352 (M^+), 309, enlarged 71
8	24.65	2-meC_{24}	2.81 ± 0.33	1.30 ± 0.24	1.38 ± 0.21	0.52 ± 0.33	352 (M^+), 337, 309
9	24.86	Z9:C_{25}*	16.3 ± 1.3	3.92 ± 1.1	37.8 ± 1.3	0.30 ± 0.19	350 (M^+), 83, 97, 111
10	24.97	Z7:C_{25}*	1.2 ± 0.52	0.47 ± 0.28	7.90 ± 0.64	nd	350 (M^+), 83, 97, 111
11	25.23	nC_{25}	17.0 ± 1.3	11.9 ± 1.1	7.02 ± 0.68	11.25 ± 4.1	352 (M^+)
12	25.80	11-meC_{25} 13-meC_{25}	0.84 ± 0.09	0.65 ± 0.18	nd	1.14 ± 0.60	366 (M^+), 168, 224 366 (M^+), 196
13	26.47	3-meC_{25}	3.77 ± 0.50	1.73 ± 0.21	4.43 ± 0.44	3.76 ± 1.1	366 (M^+), 337
14	26.94	nC_{26}	1.99 ± 0.19	1.04 ± 0.13	0.78 ± 0.15	0.25 ± 0.27	366 (M^+)
15	28.11	2-meC_{26}	3.49 ± 0.97	9.89 ± 0.35	1.45 ± 0.35	14.2 ± 4.9	380 (M^+), 365, 337
16	28.38	Z9:C_{27}*	2.47 ± 0.24	0.89 ± 0.30	6.55 ± 1.3	2.18 ± 0.91	378 (M^+), 83, 97, 111
17	28.53	Z7:C_{27}*	0.55 ± 0.16	nd	3.50 ± 1.2	nd	378 (M^+), 83, 97, 111
18	28.84	nC_{27}	6.18 ± 0.98	5.79 ± 0.18	1.85 ± 0.55	5.62 ± 1.4	380 (M^+)
19	29.50	11-meC_{27} 13-meC_{27}	0.67 ± 0.18	0.69 ± 0.23	0.85 ± 0.74	8.16 ± 6.6	379 (M-15), 168, 252 379 (M-15), 196, 224
20	30.36	3-meC_{27}	2.39 ± 0.27	2.83 ± 0.34	1.10 ± 0.36	3.81 ± 1.4	394 (M^+), 365
21	30.93	nC_{28}	0.79 ± 0.14	1.01 ± 0.15	0.42 ± 0.42	nd	394 (M^+)
22	32.10	C_{29} diene	0.48 ± 0.20	nd	0.44 ± 0.24	nd	404 (M^+), 82, 96, 109
23	32.31	2-meC_{28}	2.91 ± 0.28	13.9 ± 1.18	0.60 ± 0.27	6.92 ± 1.9	408 (M^+), 393, 365
24	32.65	Z9:C_{29}*	0.72 ± 0.20	1.91 ± 0.39	2.20 ± 0.23	5.47 ± 2.5	406 (M^+), 83, 97, 111
25	33.17	nC_{29}	2.80 ± 0.38	6.80 ± 0.50	0.72 ± 0.35	2.00 ± 1.2	408 (M^+)
26	33.89	11-meC_{29} 13-meC_{29} 15-meC_{29}	2.35 ± 0.18	11.7 ± 1.3	0.70 ± 0.23	16.08 ± 5.4	422 (M^+), 168, 280/281 422 (M^+), 196, 252 422 (M^+), 224
27	34.92	3-meC_{29}	1.83 ± 0.34	1.64 ± 0.36	0.09 ± 0.09	nd	422 (M^+), 407, 393
28	38.03	nC_{31}	0.94 ± 0.21	0.85 ± 0.22	nd	nd	436 (M^+)
29	38.77	15-meC_{31}	0.67 ± 0.21	4.67 ± 0.46	nd	1.73 ± 1.01	435 (M-15), 224, 252
30	39.41	13,17dimeC_{31}	0.29 ± 0.19	4.91 ± 0.75	nd	nd	449 (M-15),196/295, 224/267

*Double bond position and stereochemistry determined from mass spectra and retention times of the corresponding epoxides, formed by oxidation with *m*-chloroperbenzoic acid

hydrocarbons (expressed as a proportion of the total peak area of the integrated total ion chromatogram) on the dorsal surface of the elytra was similar to that sampled from other parts of the bodies of females, including ventral elytral surfaces (34.6 ± 2.5%), thoracic tergites (36.3 ± 3.0%), and abdominal sternites (34.6 ± 1.7%; means not significantly different, ANOVA $F_{3,13} = 0.91$, $P = 0.47$).

Chromatograms from SPME sampling of male elytra also showed a single dominant peak, a combination of methyl branched C_{29} compounds (Peak 26, Figure 2), which had been among several dominant peaks in chromatograms from whole body solvent extracts (Figure 1, Table 1). SPME sampling of males did not reveal any compounds that were not present in the whole-body extracts (Table 1).

Testing the activity of hydrocarbon standards

In bioassays, all male *M. robiniae* (100% of 144 trials) attempted to mate with freshly freeze-killed females, confirming that mate recognition cues were intact in the female carcasses. However, males did not respond to the same dead females after solvent extraction, demonstrating that contact sex pheromones had been removed by the solvent wash. No males (0 of 144 trials) showed any response to solvent-extracted dead females treated with pure solvent (controls). Males also showed no response to solvent-extracted female carcasses treated with a reconstructed blend of the insect-produced straight-chain alkanes, and only one male responded to a reconstructed blend of the two most abundant methyl-branched compounds found in extracts from females (Table 2). However, 56% of males showed some response to females treated with a mixture of the three most abundant monoenes, usually by aligning their bodies with the treated female carcass (Table 2). Pairwise combinations of the three monoenes elicited a significant response in males only when they included Z9:C_{25} (Table 2). When monoenes were tested individually, Z9:C_{25} elicited a full mating response in most males (attempting to couple the genitalia). A weaker response to Z9:C_{27} alone (primarily by orienting to the female; Table 2) could be due to its structural similarity with

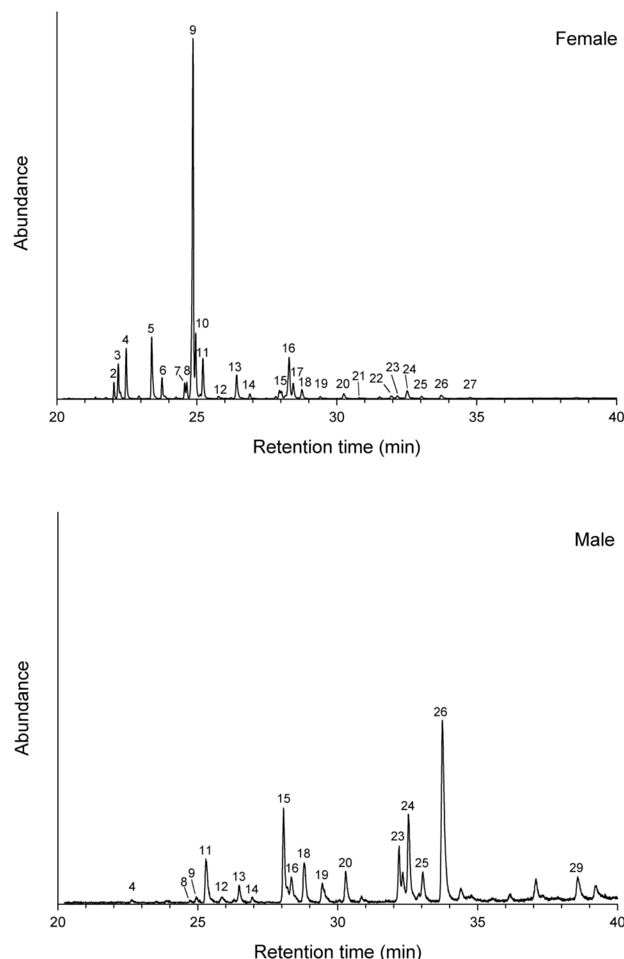

Fig. 2 Representative total ion chromatograms of cuticular hydrocarbons from an adult female (top) and male (bottom) *Megacyllene robiniae* sampled by wiping the elytra with a SPME fiber. Analysis conditions are described in Materials and methods

*Z*9:C_{25}, but the lack of synergism when the compounds were tested together suggests that *Z*9:C_{27} is not a critical component of the pheromone.

Discussion

The lack of response of male *M. robiniae* to solvent-extracted female carcasses, or dead females treated with solvent alone, indicates that contact pheromones are the primary signals used for mate recognition, with tactile or visual cues possibly playing a subordinate role. Our data suggest that *Z*9:C_{25} is a contact sex pheromone of female *M. robiniae.* Responses of males to this compound were strongest when it was applied to females as a single component (Table 2). The reduced responses obtained when *Z*9:C_{25} was combined with the other relatively abundant monoenes in extracts of females (Table 2) are difficult to explain because these same compounds apparently had no such inhibitory effect in the crude extract. It is possible that the males are sensitive to the blend ratio of these components, and the binary and ternary blends tested may have been outside the range required for optimal male responses.

Extracts from male and female *M. robiniae* were qualitatively similar, with most cuticular components occurring in both sexes (Figure 1, Table1). Quantitatively, the extracts were quite different, with extracts from males containing a greater percentage of heavier, longer chain compounds, as has been found with another species of longhorned beetle, *Xylotrechus colonus* F. (Ginzel *et al.* 2003). Sex-based differences in alkyl chain lengths of cuticular hydrocarbons, but with larger compounds predominating in females rather than males, have also been reported in the tsetse fly (Nelson & Carlson 1986) and the bark beetle *Ips lecontei* Swaine (Page *et al.* 1997).

Somewhat surprisingly, the cuticular component with sex pheromonal activity, *Z*9:C_{25}, was not specific to females, being present in smaller quantities in solvent extracts of male *M. robiniae*. Although this would seem to confound mate recognition, a similar pattern has been found in other species, with cuticular hydrocarbons that serve as contact pheromones being present in extracts of the cuticles of both sexes, but quantitatively dominant in the appropriate sex. For example, components of the sex pheromone of the dipteran *Musca autumnalis* De Geer (*Z*13:C_{27}, *Z*13:C_{29}, and *Z*14:C_{29}) were more abundant on females than on males (Uebel *et al.* 1975). Similarly, a component of the female contact sex pheromone of the cerambycid *Xylotrechus colonus* (nC_{25}) also is present in extracts of males, but in relatively small quantities (Ginzel *et al.* 2003).

One of the most interesting points to emerge from this study was the marked difference in the relative amounts of compounds recovered by solvent extraction versus SPME wipe sampling of the cuticle. The classical method of solvent extraction strips the entire wax layer from the cuticle, and the extract also may be contaminated with internal body lipids and exocrine gland secretions. In contrast, wiping the SPME fiber over the cuticle primarily samples the outer surface of the wax layer only, and the sample should not be contaminated with internal lipids. In the case of *M. robiniae*, sampling female cuticular hydrocarbons by SPME revealed that the contact pheromone, *Z*9:C_{25}, was by far the most abundant hydrocarbon on the surface of the wax layer, where it would be readily accessible to the antennae of males. Although this compound also was present in hexane extracts of males, it comprised a negligible proportion of the SPME samples of the male wax layer. These findings suggest that male *M. robiniae* distinguish between the sexes by contact chemoreception based on presence or absence of *Z*9:C_{25}. Cues involved in species and/or mate recognition may also be provided by other quantitatively abundant compounds on the surface of the cuticular wax layer of males, including 2-meC_{26} (peak 15 in Figure 2, Table 1), and the internally branched C_{29} compounds (peak 26). We conclude that for these and other insects, wipe sampling by SPME may yield a more representative sample of the components of the cuticular wax layer that are actually encountered by a male's antennae, and so may provide a more readily interpretable profile of potential semiochemicals present in the wax layer.

Table 2 Response of male *Megacyllene robiniae* to solvent-washed dead females to which hydrocarbon standards had been applied in dosages of 0.2 FE (n = 16 different males per compound or blend). Behavior of responding males was categorized according to the natural sequence of mating behavior steps of 1) orientation toward female (turns toward female), 2) arrestment, 3) male aligns body with female, 4) male mounts female and attempts to couple genitalia. Percentages of males responding to treatments were compared to their responses to solvent-treated controls using χ^2 tests

Compound(s)	No. of males responding (%)	χ^2 statistic(P)	No. of males responding per step in the behavioral sequence			
			Step 1	Step 2	Step 3	Step 4
Straight-chain alkanes (nC_{23}, nC_{24}, nC_{25}, nC_{26})	0 (0%)	0 ($P = 1$)	0	0	0	0
Methyl-branched alkanes (3me-C_{23}, 3me-C_{25})	1 (6%)	1.0 ($P > 0.1$)	1	1	1	0
Monoenes ($Z9$:C_{23}, $Z9$:C_{25}, $Z9$:C_{27})	9 (56%)	12.5 ($P < 0.001$)	9	8	7	1
$Z9$:C_{23} + $Z9$:C_{27}	3 (19%)	3.3 ($P > 0.1$)	3	2	2	0
$Z9$:C_{23} + $Z9$:C_{25}	9 (56%)	12.5 ($P < 0.001$)	9	3	2	0
$Z9$:C_{25} + $Z9$:C_{27}	12 (75%)	19.2 ($P < 0.001$)	12	12	10	4
$Z9$:C_{23}	1 (6%)	1.0 ($P > 0.1$)	1	1	1	0
$Z9$:C_{25}	15 (94%)	28.2 ($P < 0.001$)	15	15	15	11
$Z9$:C_{27}	7 (44%)	9.0 ($P < 0.01$)	7	2	1	0

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References

Bland JM, Osbrink WLA, Cornelius ML, Lax AR, and Vigo CB (2001) Solid-phase microextraction for the detection of termite cuticular hydrocarbons. J Chromatogr A 932:119–127

Blomquist GJ, Tillman-Wall JA, Guo L, Quilici DR, Gu P, Schal C (1996) Hydrocarbon and hydrocarbon derived sex pheromones in insects: biochemistry and endocrine regulation. Pp. 317–351 *in* Stanley-Samuelson DW, Nelson DR (eds) Insect Lipids: Chemistry, Biochemistry and Biology, USA, NE-Lincoln: University of Nebraska Press

Coll JC, Bowden BF (1986) The application of vacuum liquid chromatography to the separation of terpene mixtures. J Nat Prod 49:934–936

Fukaya M, Yasuda T, Wakamura S, Honda H (1996) Reproductive biology of the yellow-spotted longicorn beetle, *Psacothea hilaris* (Pascoe) (Coleoptera: Cerambycidae). III. Identification of contact sex pheromone on female body surface. J Chem Ecol 22:259–270

Fukaya M, Wakamura S, Yasuda T, Senda S, Omata T, Fukusaki SE (1997) Sex pheromonal activity of geometric and optical isomers of synthetic contact pheromone to males of the yellow-spotted longicorn beetle, *Psacothea hilaris* (Pascoe) (Coleoptera: Cerambycidae). Appl Entomol Zool 32:654–656

Fukaya M, Akino T, Yasuda T, Wakamura S, Satoda S, Senda S (2000) Hydrocarbon components in contact sex pheromone of the white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae) and pheromonal activity of synthetic hydrocarbons. Entomol Science 3: 211–218

Gibbs AG (1998) Water-proofing properties of cuticular lipids. Am Zool 38:471–482

Galford JR (1984) The locust borer. Forest Insect and Disease Leaflet 71. USA, WA-Washington, DC: U.S. Department of Agriculture, Forest Service

Ginzel MD, Blomquist GJ, Millar JG, Hanks LM (2003) The role of contact pheromones in mate location and recognition in *Xylotrechus colonus*. J Chem Ecol 29:533–545

Ginzel MD, Hanks LM (2003) Contact pheromones as mate recognition cues of four species of longhorned beetles (Coleoptera: Cerambycidae). J Insect Behav 16:181–187

Kim G, Takabayashi J, Takahashi S, Tabata K (1993) Function of contact pheromone in the mating behavior of the cryptomeria bark borer, *Semanotus japonicus* Lacordaire (Coleoptera: Cerambycidae). Appl Entomol Zool 28:525–535

Liebig J, Peeters C, Oldham NJ, Markstadter C, Hölldobler B (2000) Are variations in cuticular hydrocarbons of queen and workers a reliable signal of fertility in the ant *Harpegnathos saltalor*? Proc Natl Acad Sci 97:4124–4131

Moneti G, Dani FR, Pieraccini G, Turillazzi S (1997) Solid-phase microextraction of insect epicuticular hydrocarbons for gas chromatographic mass spectrometric analysis. Rapid Commun Mass Spectrom 11:857–862

Monnin T, Malosse C, Peeters C (1998) Solid-phase microextraction and cuticular hydrocarbon differences related to reproductive activity in the queenless ant *Dinoponera quadriceps*. J Chem Ecol 24:473–490

Nelson DR (1993) Methyl-branched lipids in insects. Pp. 271–315 *in* Stanley-Samuelson DW, Nelson DR (eds) Insect Lipids: Chemistry, Biochemistry, and Biology. USA, NE-Lincoln: University of Nebraska Press

Nelson DR, Blomquist GJ (1995) Insect waxes. Pp.1–90 *in* Hamilton RJ (ed.) Waxes: Chemistry, Molecular Biology, and Function. GB-Dundee: The Oily Press

Nelson DR, Carlson DA (1986) Cuticular hydrocarbons of the tsetse flies *Glossina morsitans morsitans*, *G. austeni*, and *G. pallidipes*. Insect Biochem 16:403–416

Page M, Nelson LJ, Blomquist GJ, Seybold SJ (1997) Cuticular hydrocarbons as chemotaxonomic characters of pine engraver beetles (*Ips* spp.) in the *grandicollis* subgeneric group. J Chem Ecol 23:1053–1099

Peeters C, Monnin T, Malosse C (1999) Cuticular hydrocarbons correlated with reproductive status in a queenless ant. Proc R Soc Lond B 266:1323–1327

Roux E, Sreng L, Provost E, Roux M, Clement JL (2002) Cuticular hydrocarbon profiles of dominant versus subordinate male *Nauphoeta cinerea* cockroaches. J Chem Ecol 28: 1221–1235

Sledge MJ, Moneti G, Pieraccini G, Turillazzi S (2000) Use of solid phase microextraction in the investigation of chemical communication in social wasps. J Chromatogr A 873:73–77

Sokal RR, Rohlf FJ (1995) Biometry: 3rd ed. USA, NY-New York: Freeman
Solomon JD (1995) Guide to Insect Borers in North American Trees and Shrubs. Agric Handbk 706, U.S. Dept of Agriculture, Forest Service, Washington D.C
Tentschert J, Bestmann HJ, Heinze J (2002) Cuticular compounds of workers and queens in two *Leptothorax* ant species – a comparison of results obtained by solvent extraction, solid sampling, and SPME. Chemoecology 12:15–21
Turillazi S, Sledge MF, Monetti G (1998) Use of a simple method for sampling cuticular hydrocarbons from live social wasps. Ethol Ecol Evol 10:293–297
Uebel EC, Sonnet PE, Miller RW, Beroza M (1975) Sex pheromone of the face fly, *Musca autumnalis* De Geer (Diptera: Muscidae). J Chem Ecol 1:195–202
Wang Q (1998) Evidence for a contact female sex pheromone in *Anoplophora chinensis* (Förster) (Coleoptera: Cerambycidae: Lamiinae). Coleopt Bull 52:363–368

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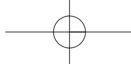
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