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General Food Semiochemicals Attract Omnivorous German Cockroaches, *Blattella germanica*

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ABSTRACT: Stale beer and peanut butter are effective baits for the German cockroach (GCRs), *Blattella germanica* (L.) (Dictyoptera: Blattellidae). In still-air arena olfactometer experiments it was previously shown that headspace volatile extracts of peanut butter and solvent extract of beer attract male GCRs. The objective of this study was to identify the semiochemicals that mediate attraction of GCRs to these sources. Coupled gas chromatographic−electroantennographic detection (GC-EAD) and GC−mass spectrometric (MS) analyses of these attractive extracts, or fractions thereof, and of synthetic standards revealed many candidate semiochemicals. Elaborate olfactometer experiments determined that 1-hexanol from peanut butter, and ethanol and 2, 3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) from beer, are the key semiochemicals of these food sources. 1-Hexanol is a well-known headspace volatile of decomposing lipids, ethanol conveys food fermentation, and DDMP with a caramel-type flavor has been found in several types of food. By responding to these rather general food-derived compounds, the omnivorous GCRs appear to exploit semiochemicals that indicate the presence of various food components, such as lipids and carbohydrates. Synthetic equivalents of these semiochemicals may be formulated as baits or be added to, and thus enhance the attractiveness of, natural food sources as trap or insecticidal baits.

KEYWORDS: Peanut butter, beer, 1-hexanol, ethanol, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one

INTRODUCTION

The German cockroach (GCR), *Blattella germanica* (L.) (Dictyoptera: Blattelidae), is one of the most significant urban and food-associated pests worldwide.[1] Movement of GCRs between organic waste and food materials allows them to acquire, carry, and transfer pathogens of human illnesses,[1] such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp.[2,3] Exposure to cockroach-derived allergenic proteins in homes is associated with allergic disease and asthma, particularly in inner-city children.[4] However, extensive sanitation and cockroach control can greatly reduce cockroach allergens in household dust.[5]

Effective attractants that lure GCRs to traps and insecticide baits (compositions that induce insects to make oriented movements toward the source) can significantly enhance successful abatement programs.[6] Because pheromones are typically effective attractants,[7] previous studies were undertaken to understand the pheromonal communication system of GCRs. Aggregation behavior of female, male, and nymph GCRs is mediated by both attractant and arrestant components. Sakuma and Fukami[8] isolated and identified ammonia and 12 amines including 1-dimethylamino-2-methyl-2-propanol from frass-contaminated filter paper that elicits attraction. Sakuma and Fukami[9] also isolated and identified the two major arrestant components blattelastanoside-A and blattelastanoside-B of the GCR aggregation pheromone. The sex pheromone of GCRs consists of the nonvolatile components 3, 11-dimethyl-2-nonacosanone, 29-hydroxy-3,11-dimethyl-2-nonacosanone, 29-oxo-3,11-dimethyl-2-nonacosanone, and 3,11-dimethyl-2-heptacosanone on the females' cuticular surface[10] and a volatile component[11] that was only recently identified as gentisyl quinone isovalerate (blattellaquinone[12]). In the final phase of courtship, the male secretes from his abdominal tergal glands a complex assortment of sugars, phospholipids, cholesterol, and amino acids that elicits a feeding response from the female and brings her to the precopulatory position.[13]

There is also evidence for auditory communication signals and cues. Females and nymphs produce click-type sound pulses of ca. 10 ms duration and peak frequencies of 7, 9, 11, and 14 kHz that attract nymphs.[14] Moreover, in groups, GCRs wing fan, and gravid females utilize auditory cues associated with wing-fanning behavior when they decide whether or not to enter a shelter.[15]

Neither synthetic pheromones nor sound has yet been deployed intensively in abatement programs. This may be due to their offensive smell (amines), complex structure (blattellastanosides), or gender- or stage-specific attractiveness (blattellaquinone; sound clicks) or because findings are still too recent (blattellaquinone; wing-fanning sound) to have been adopted by the pest management industry. Alternatively, food-based attractants offer cheaper and possibly equally effective trap or insecticidal baits.

Although GCRs are considered omnivores, the nutritional composition of their prior meal may affect their selection of subsequent meals.[16] This may explain why the list of food types and food-derived semiochemicals (message-bearing chemicals) for attraction of GCRs contains all constituents of a balanced diet, including carbohydrates, proteins, lipids, fruits, and vegetables.

A plethora of "home recipes" have been suggested as baits for attracting GCRs in human dwellings (see refs 17−19 and references cited therein). According to these recipes effective

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Table 1. Stimuli Tested in Still-Air Arena Olfactometer Experiments 1−26

		stimuli tested	
expt[a]	n[b]	stimulus 1	stimulus 2
1	9	peanut butter[c] (4 g)	unbaited
2	10	Porapak Q extract (pentane; 105 μL) of peanut butter volatiles (373 GHE)[d]	pentane (105 μL)
3	12	beer (4 mL)[e]	water (4 mL)
4	12	Porapak Q extract (pentane; 22 μL) of beer (24 MLHE)[f]	pentane (22 μL)
5	9	ether/methanol extract (10:1; 4 mL) of beer (4 mL)	ether/MeOH (10:1; 4 mL)
6	9	silica fractions 1−5 in pentane/ether (10 mL) of ether/methanol extract (10:1; 4 mL) of beer (4 mL)	pentane/ether (4 mL)
7	9	silica fractions 1−3 in pentane/ether (6 mL) of ether/methanol extract (10:1; 4 mL) of beer (4 mL)	pentane/ether (4 mL)
8	9	silica fractions 4−5 in pentane/ether (4 mL) of ether/methanol extract (10:1; 4 mL) of beer (4 mL)	pentane/ether (4 mL)
9	14	synthetic blend (SB-1)[g] in ether (54 μL)	ether (54 μL)
10	16	SB-1 minus 2-(4-hydroxyphenyl)ethanol	ether (54 μL)
11	12	SB-1 minus DDMP	ether (54 μL)
12	10	SB-1 minus 2-phenylethanol minus 2-(4-hydroxyphenyl)ethanol	ether (54 μL)
13	10	ethanol (200 μL) plus DDMP (4 μg) in MeCN (16 μL)	MeCN (16 μL)
14	8	DDMP (4 μg) in MeCN (16 μL)	MeCN (16 μL)
15	10	ethanol (200 μL)	unbaited
16	10	beer (4 mL)	water (4 mL)
17	15	synthetic blend (SB-2)[h] in pentane (103 μL)	pentane (103 μL)
18	15	SB-2 minus aldehydes	pentane (103 μL)
19	15	SB-2 minus pyrazines minus 1-hexanol	pentane (103 μL)
20	11	SB-2 minus aldehydes	pentane (103 μL)
21	11	SB-2 minus aldehydes minus 1-hexanol	pentane (103 μL)
22	6	SB-2 minus aldehydes minus pyrazines	pentane (103 μL)
23	7	ethanol (200 μL) plus DDMP (4 μg) plus 1-hexanol (0.6 μg) in MeCN/pentane (16 μL/75 μL)	MeCN/pentane (16 μL/75 μL)
24	7	ethanol (200 μL) plus DDMP (4 μg) in MeCN (16 μL)	MeCN (16 μL)
25	7	1-hexanol (0.6 μg) in pentane (75 μL)	pentane (75 μL)
26	7	ethanol (200 μL)	unbaited

[a] Experiments 1−2, 3−4, 5−8, 9−12, 13−16, 17−19, 20−21, and 23−26 were run concurrently. [b] n = number of replicates. [c] Great Value Peanut Butter, Coquitlam, BC, Canada. [d] GHE, g-h equivalent; 1 GHE = amount of volatiles released from 1 g of peanut butter during 1 h. [e] MLHE, 1 mL-h equivalent; 1 MLHE = amount of volatiles released from 1 mL of beer (Pale Ale, Okanagan Spring Brewery, BC, Canada) during 1 h. [f] Pale Ale (see footnote *e*). [g] SB-1, synthetic blend 1 [ethanol (200 μL), 2-phenylethanol (200 μg), 2-(4-hydroxyphenyl)ethanol (40 μg), and DDMP (4 μg)]. [h] SB-2= Synthetic blend 2 [1-hexanol (0.6 μg), hexanal (0.05 μg), heptanal (0.1 μg), nonanal (0.4 μg), 2,5-dimethylpyrazine (0.8 μg), 2-ethyl-5-methylpyrazine (0.7 μg), and 2-ethyl-3,5-dimethylpyrazine (0.25 μg)].

baits are white flour, white bread, oatmeal, cocoa, rice bran, pregelatinized tapioca, wheat starch, corn oil, and various corn products, such as corn meal and corn distiller's dried grains with solubles obtained from nonbeverage alcohol production. Some food types are generally well accepted as effective baits for GCRs. These include pet or dog food, stale beer, and peanut butter.

Baits based on peanut butter are widely used by pest management professionals, and stale beer is a well-known and recommended home recipe bait for GCRs.[17] In olfactometer experiments, peanut butter and stale beer were confirmed to strongly attract GCRs.[20] If the essential semiochemicals of these food sources were known, they could be used to enhance or replace attractants such as peanut butter currently used in traps, thus significantly contributing to successful GCR abatement programs. Here we report the identification of semiochemicals that lure GCRs to beer and peanut butter.

MATERIALS AND METHODS

Experimental Insects. A colony of GCRs was established with nymphs and adults obtained from the insectary of SC Johnson & Son (Racine, WI). The colony was supplemented with specimens captured in an apartment building in Vancouver (BC, Canada). Insects were reared in the Insectary Annex of Simon Fraser University in plexiglass cages (30 × 60 × 45 cm; W × L × H) fitted with two mesh-covered openings for ventilation. The cages were maintained at 25 ± 1 °C and 40−70% relative humidity, with a photoperiod of L14/D10. Shelter was provided by crumpled paper towels and panels of narrowly spaced particle board. The diet consisted of Safeway Select dog food, apple slices, and water. Males used in experiments were up to 4 weeks old. Each specimen was bioassayed only once and placed in a specific rearing cage after the bioassay.

Headspace Volatiles from Peanut Butter and Beer. Great Value peanut butter (100 g; Wal-Mart, Mississauga, ON, Canada) was placed into a glass chamber (15.5 i.d. × 20 cm), and charcoal-filtered air

Table 2. Compounds in Headspace Volatile or Solvent Extracts of Beer That Elicited Antennal Responses from Male German Cockroaches, *Blattella germanica*, in Gas Chromatographic−Electroantennographic Detection Analyses

compd no.[a]	compound name	RI[b]	ng/μL[d]	source[e]	supplier	purity (%)
1	2-phenylethanol	1116	186	HS/SE	Fluka[f]	99
2	DDMP[c]	1145	1	SE	SFU[g]	
3	octanoic acid	1168	1	HS	Aldrich[h]	98
4	ethyl octanoate	1196	40	HS	SFU[i]	95
5	decanal	1207	3	HS	Aldrich[h]	99
6	phenylethyl acetate	1258	54	HS	SFU[j]	
7	1-decanol	1275	3	HS	Aldrich[h]	98
8	γ-nonalactone	1362	1	HS	Bedoukian[k]	98
9	ethyl decanoate	1395	12	HS	SFU[l]	
10	2-(4-hydroxyphenyl)ethanol	1424	10	SE	Aldrich[h]	98

[a] Numbers as in Figure 2. [b] RI, retention index[23] on a DB-5 column. [c] 2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one. [d] Amount (ng) per microliter in headspace volatile extract or solvent extract (compounds **2** and **10**). [e] HS, headspace; SE, solvent extract. [f] Fluka Chemie, Buchs, Switzerland. [g] Synthesized according to Kim and Baltes.[26] [h] Sigma-Aldrich, Oakville, ON, Canada. [i] Synthesized by esterification of octanoic acid with ethanol. [j] Synthesized by esterification of acetic acid with 2-phenylethanol. [k] Bedoukian Research Inc., Danbury, CT. [l] Synthesized by esterification of decanoic acid with ethanol.

was drawn at 1 L/min for 71 h through the chamber and a glass tubing (14 × 1.3 cm o.d.) containing 500 mg of Porapak-Q (50−80 mesh, Waters Associates Inc., Milford, MA). Volatiles were eluted from Porapak-Q with 2 mL of pentane. The acquisition of headspace volatiles from fresh beer (100 mL; Pale Ale, Okanagan Spring Brewery, Delta, BC, Canada) was similar except that the beer was retained in a 250 mL Erlenmeyer flask and aerated for 24 h.

To clean Porapak-Q volatile traps, they were rinsed with 2 mL each of pentane and ether and purged with N_2 at 55 °C for 10 min.

Extracts of Beer. Porapak-Q extract of beer had a smell only remotely similar to beer, prompting concern that one or more essential semiochemicals were not, or not sufficiently, captured on Porapak-Q. Thus, 10-mL aliquots of beer (<1 day old) were also extracted with a 10-mL ether/methanol (9:1) mixture. After the solvent had been added to the beer, the mixture was gently shaken for 10 s, after which time the supernatant solvent was withdrawn.

Behavioral Evidence for the Presence of Semiochemicals in Headspace Volatile or Solvent Extracts of Peanut Butter and Beer. Aliquots of Porapak-Q or solvent extracts were pipetted onto a braided cotton roll (8 × 1 cm; Richmond Dental, Charlotte, NC) retained in a Petri dish (5 cm diameter); equivalent amounts of solvent were applied onto a control cotton roll. Both treatment and control Petri dishes were covered with mesh that allowed volatiles to emanate but prevented access of GCRs to the source. A treatment or control Petri dish was placed inside an electrical trap modified according to the method of Mistal et al.[14] It consisted of an open aluminum can (15.8 × 16 cm, D × H) designed such that a GCR dropped into the trap once a leg touched an insulated copper ribbon (first electrode) while the other legs were on the inside wall of the can (second electrode), resulting in the completion of a 16-V circuit, briefly stunning and trapping the insect. Traps were placed at opposite quadrants of the plexiglass (118 × 39.5 cm) arena 10 cm from the wall. Mounted above the arena's lid was a 63.5-mm fluorescent light (warm white brightstick 33; General Electric, Cleveland, OH) programmed to produce a L14/D10 photoperiod. Temperature and relative humidity during experiments were comparable to those in the Insectary Annex (see above). Experimental replicates were started at the onset of the scotophase (set to 3:00 p.m.) by placing a paper-lined glass tube (40 × 2 cm) containing 20 (±1) 2-day-starved but water-provisioned males in the middle of the arena and allowing them to exit the tube and to forage for ∼21 h.

In each of two-choice experiments 1−4 and 5−26 (see below), treatment and control stimuli were randomly assigned to each position. Following each replicate, each trap was moved clockwise to the adjacent quadrant, and traps and arenas were cleaned with Purell hand sanitizer (Pfizer Canada Inc., Markham, ON, Canada) and left to aerate for ∼1 h.

Concurrent experiments 1 and 2 (n = 9−13) and 3 and 4 (n = 12) tested peanut butter versus a no-bait control (experiment 1), aliquots of Porapak-Q peanut butter headspace volatile extract versus a pentane solvent control (experiment 2), beer versus a water control (experiment 3), and aliquots of Porapak-Q beer headspace volatile extract versus a pentane solvent control (experiment 4; Table 1).

Proportions of insects responding (trap-captured) in experiments 1−4 and 5−26 (see below) were analyzed by the Wilcoxon test.[21]

Identification of Candidate Semiochemicals in Headspace or Solvent Extracts of Beer and Peanut Butter. Aliquots of Porapak-Q headspace volatile extracts of peanut butter and beer as well as solvent extract of beer (see above), or fractions thereof eluted from silica ("silica fractions"; see below), were analyzed by coupled gas chromatographic−electroantennographic detection (GC-EAD)[22] and GC−mass spectrometry (MS). GC-EAD analyses employed a Hewlett-Packard (HP) 5890A gas chromatograph equipped with a GC column (30 m × 0.25 or 0.32 mm i.d.) coated with DB-5, DB-23, or DB-210 (J&W Scientific, Folsom, CA). For GC-EAD recordings, the base of an antenna was carefully removed from an insect's head and placed into the opening of a glass capillary electrode filled with saline solution. The tip of the antenna was then removed by spring microscissors (Fine Science Tools Inc., North Vancouver, BC, Canada), and the tip of the antenna with the most distal section lacking was placed into the opening of a second electrode. Ethanol as a major constituent of beer (4.3%), and as an obvious candidate semiochemical, was tested by subjecting headspace volatiles of HPLC-grade ethanol to GC-EAD analysis in split mode.

Compounds that elicited antennal responses were identified by full-scan electron-impact mass spectrometry with a Varian Saturn 2000 Ion Trap GC-MS fitted with a DB-5 MS column (30 m × 0.25 mm; film thickness = 0.25 μm) (J&W Scientific) and retention index calculations.[23] The identification of antennal stimulatory compounds was confirmed by comparing their GC retention times and mass spectra with those reported in the literature [decanal, nonanal, 2-phenylethanol, ethyl octanoate, and 2-phenylethyl acetate;[24] hexanal, heptanal, γ-nonalactone, 1-hexanol, 1-decanol, and ethyl decanoate;[25] 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP);[26] octanoic acid;[27] 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, and 2-ethyl-3,5-dimethylpyrazine[28,29]] and with those of authentic standards (see below). The amounts of EAD-active compounds were quantified by comparing their GC peak integration with those of known amounts of authentic standards.

Table 3. Compounds in Headspace Volatiles of Peanut Butter (Great Value Peanut Butter; Wal-Mart, Coquitlam, BC, Canada) That Elicited Antennal Responses from Male German Cockroaches, *Blattella germanica*, in Gas Chromatographic−Electroantennographic Detection Analyses

compd no.[a]	compd name	RI[b]	ng/μL	supplier	purity (%)
11	hexanal		0.5	Aldrich[c]	98
12	1-hexanol	870	6	Aldrich[c]	98
13	heptanal	895	1	Aldrich[c]	95
14	2,5-dimethylpyrazine	908	8	Aldrich[c]	98
15	2-ethyl-5-methylpyrazine	999	7	Penta[d]	99
16	2-ethyl-3,5-dimethylpyrazine	1077	2	Arcos[e]	99
17	nonanal	1105	4	Aldrich[c]	95
*	1,4-dichlorobenzene	1015	1	Aldrich[c,g]	98
**	2,3-dimethyl-5-(2-propenyl)pyrazine	1173	0.25	SFU[f,g]	95

[a] Numbers or asterisks as in Figure 3. [b] Retention index[23] on a DB-5 column. [c] Sigma-Aldrich, Oakville, ON, Canada. [d] Penta Manufacturing, Livingston, NJ. [e] Arcos Organics, Morris Plains, NJ. [f] Synthesized by allylation of 2,3-dimethylpyrazine with allyllithium.[28] [g] 1,4-Dichlorobenzene and 2,3-dimethyl-5-(2-propenyl)pyrazine were deemed to be a contaminant and to be only tentatively identified, respectively, and thus they were not included in bioassays.

To determine the essential semiochemicals in beer, 4-mL aliquots of ether/methanol (9:1) beer extracts were concentrated to near dryness (10 μL residue) under a stream of nitrogen and reconstituted with pentane. The reconstituted extract was then fractionated on silica gel 60 (230−400 mesh, E. Merck, Darmstadt, Germany) (0.5 g) in a glass column (14 × 0.5 cm i.d.). After the silica gel had been prerinsed with pentane, the extract was applied and compounds were eluted with 2 mL each of pentane/ether (100:0, 90:10, 75:25, 50:50, and 0:100), generating five fractions that contained analytes of increasing polarity.

Chemicals. Synthetic standards needed for identification and behavioral experiments were purchased from suppliers listed in Tables 2 and 3.

Syntheses. 2-Phenylethyl acetate was produced by acetylation of the corresponding alcohol. Ethyl octanoate and ethyl decanoate were synthesized by esterification of octanoic and decanoic acid, respectively, with ethanol. DDMP was synthesized according to the procedure of Kim and Baltes.[26] 2,3-Dimethyl-5-(2-propenyl)pyrazine was synthesized by allylation of 2,3-dimethylpyrazine with allyllithium,[28] the latter being generated in situ from lithium and allylphenyl ether (Aldrich) according to the method of Eisch and Jacobs.[30]

DDMP was purified (>95%) by high-performance liquid chromatography (HPLC), employing a Waters LC 626 HPLC equipped with a Waters 486 variable-wavelength UV−visible detector set to 210 nm, HP Chemstation Software (rev. A. 07. 01), and a reverse-phase Phenomenex Synergi column (80 Å, 4 μm; 4.6 × 250 nm) eluted with 0.8 mL/min of acetonitrile/water (1:1).

Testing of Candidate Semiochemicals. Employing the same general experimental protocol as described above, parallel-run experiments 5−8 (n = 9 each) tested beer extract versus a water control (experiment 5), five silica fractions of beer extract recombined (experiment 6), fractions 1−3 (experiment 7), or polar fractions 4 and 5 (experiment 8) (Table 1).

Considering the attractiveness of all recombined fractions and of polar fractions 4 and 5, concurrent experiments 9−12 tested a synthetic blend (SB-1) of all EAD-active components in the polar fractions [ethanol, 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and DDMP] (experiment 9) and blends lacking specific components, such as 2-(4-hydroxyphenyl)ethanol (experiment 10) or DDMP (experiment 11), or lacking both 2-(4-hydroxyphenyl)ethanol and 2-phenylethanol (experiment 12). Concurrent experiments 13−16 retested the attractive blend of ethanol and DDMP (experiment 13) as a positive control, each compound alone (experiments 14 and 15), or beer as a second positive control (experiment 16).

Concurrent experiments 17−19 were designed to determine essential semiochemical(s) in peanut butter. Experiment 17 tested a synthetic blend (SB-2) of all antennal stimulatory compounds, including one alcohol (1-hexanol), three aldehydes (hexanal, heptanal, and nonanal), and three pyrazines (2,5-dimethyl-; 2-ethyl-5-methyl-; and 2-ethyl-3,5-dimethyl-). Experiments 18 and 19 tested SB-2 lacking specific groups of organic molecules, such as aldehydes (experiment 18) or both 1-hexanol and pyrazines (experiment 19). Concurrent experiments 20 and 21 retested as a positive control the attractive blend of SB-2 lacking aldehydes (experiment 20) and SB-2 lacking both aldehydes and 1-hexanol (experiment 21). Experiment 22 tested SB-2 lacking both aldehydes and pyrazines.

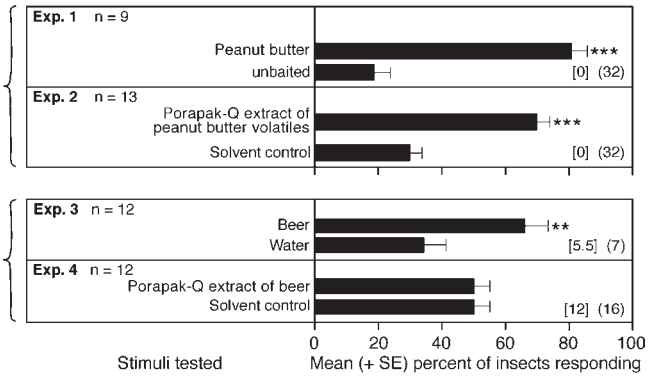

Figure 1. Percentage of male *Blattella germanica* responding in two-choice arena olfactometer experiments 1−4 (Table 1) to peanut butter, beer, or their respective headspace volatile extracts. In each experiment, the Wilcoxon T value is reported in brackets, the number in parentheses represents the percentage of nonresponding insects, and an asterisk (*) indicates a statistically significant preference for the particular test stimulus (Wilcoxon rank sum test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Experiments grouped by brackets were run concurrently; n = number of replicates.

The last set of experiments, 23−26, was designed to explore interactions between key semiochemicals of beer (ethanol and DDMP) and peanut butter (1-hexanol). Specifically, experiments 23−26 tested a three-component blend of 1-hexanol, ethanol, and DDMP (experiment 23) and a two-component blend of ethanol and DDMP (experiment 24) as well as 1-hexanol (experiment 25) and ethanol (experiment 26) singly.

RESULTS

Evidence for Semiochemicals in Headspace Volatile or Solvent Extracts of Beer and Peanut Butter. In concurrent experiments 1−2 and 3−4, peanut butter (experiment 1), Porapak Q extract of peanut butter headspace volatiles (experiment 2), and beer (experiment 3) significantly attracted male GCRs,

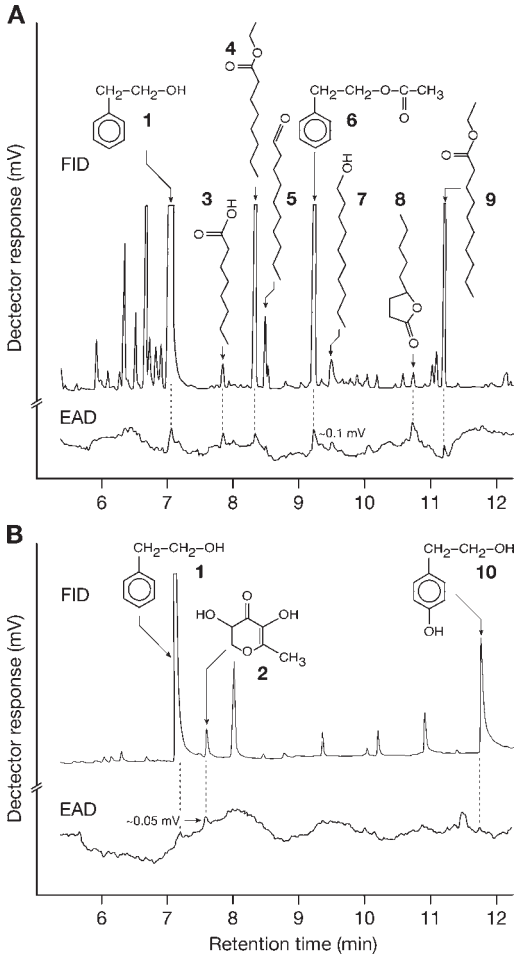

Figure 2. Representative recordings ($n = 3$) of flame ionization detector (FID) and electroantennographic detector (EAD, male *Blattella germanica* antenna) responses to aliquots of (A) Porapak Q headspace volatile extract of beer and (B) solvent extract of beer. Further information of antennal stimulatory compounds **1**−**10** is provided in Table 2. Chromatography: DB-5 column; splitless injection; temperature of injection port and FID, 240 °C; temperature program, 50 °C (1 min), 10 °C min^{-1} to 280 °C. Compounds that consistently elicited antennal responses are associated with dotted lines.

whereas Porapak Q extract of beer headspace volatiles (experiment 4) was not attractive (Figure 1).

GC-EAD and GC-MS Analyses of Semiochemical Extracts. GC-EAD and GC-MS analyses of Porapak Q extracts of beer headspace volatiles (Figure 2A) revealed two alcohols (2-phenylethanol and 1-decanol), three esters (ethyl octanoate, ethyl decanoate, and 2-phenylethyl acetate), one acid (octanoic acid), one aldehyde (decanal), and one lactone (γ-nonalactone) that elicited consistently responses from female GCR antennae. GC-EAD and GC-MS analyses of solvent extract of beer (Figure 2B) revealed three antennal stimulatory compounds [2-phenylethanol, DDMP, and 2-(4-hydroxyphenyl)ethanol], which were all present in the polar silica fractions 4 and 5 (see below). Ethanol, which was analyzed separately, was also EAD-active. In GC-EAD and GC-MS analyses of peanut butter headspace volatile extracts (Figure 3), one alcohol (1-hexanol), three aldehydes (hexanal, heptanal, and nonanal), and three pyrazines (2,5-dimethyl-; 2-ethyl-5-methyl-; and 2-ethyl-3,5-dimethyl-) elicited responses from female GCR antennae (Table 3). The amounts of all EAD-active compounds

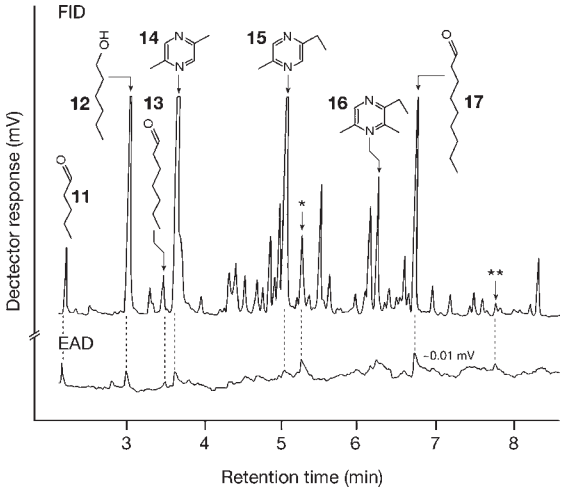

Figure 3. Representative recording ($n = 3$) of flame ionization detector (FID) and electroantennographic detector (EAD, male *Blattella germanica* antenna) responses to aliquots of Porapak Q headspace volatile extract of peanut butter. Further information on antennal stimulatory compounds **11**−**17** as well as * and ** is provided in Table 3. Chromatography was as described in the caption of Figure 2. Compounds that consistently elicited antennal responses are associated with dotted lines.

were quantified by comparing their GC peak integration with those of known amounts of authentic standards.

Testing of Candidate Semiochemicals. In concurrent arena olfactometer experiments 5−8, solvent extract of beer (experiment 5) and all recombined silica fractions of solvent-extracted beer (experiment 6) as well as polar fractions 4 and 5 (experiment 8) significantly attracted female GCRs, whereas nonpolar fractions 1−3 (experiment 7) were not attractive (Figure 4). In experiment 9, a synthetic blend (SB-1) of all EAD-active compounds in fractions 4 and 5 [2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, DDMP, and ethanol] significantly attracted females (Figure 4), as did SB-1 lacking 2-(4-hydroxyphenyl)ethanol (experiment 10) or SB-1 lacking both 2-(4-hydroxyphenyl)ethanol and 2-phenylethanol (experiment 12). In experiment 11, however, SB-1 lacking DDMP was not attractive (Figure 4). In parallel-run experiments 13−16, the two-component blend of ethanol and DDMP (experiment 13), ethanol (experiment 15), or beer (experiment 16) significantly attracted females (Figure 4), whereas the single-component lure of DDMP (experiment 14) did not.

In concurrent experiments 17−19, a synthetic blend (SB-2) of all antennal stimulatory peanut butter headspace volatiles (Table 3) (experiment 17) and SB-2 lacking aldehydes (experiment 18) were significantly more effective than a solvent control in attracting female GCRs (Figure 5), whereas in experiment 19 SB-2 lacking both pyrazines and 1-hexanol had no attractiveness. In concurrent experiments 20 and 21, SB-2 lacking aldehydes was again significantly attractive (experiment 20), whereas SB-2 lacking both aldehydes and 1-hexanol was not (experiment 21), implicating 1-hexanol as a key semiochemical. In experiment 22, SB-2 lacking both aldehydes and pyrazines (= 1-hexanol alone) was significantly more effective than a solvent control in attracting female GCRs, confirming 1-hexanol as a key semiochemical of peanut butter.

In concurrent experiments 23−26 (Figure 6) that explored potential interactions between semiochemicals from peanut butter and beer, the three-component blend of ethanol, 1-hexanol, and

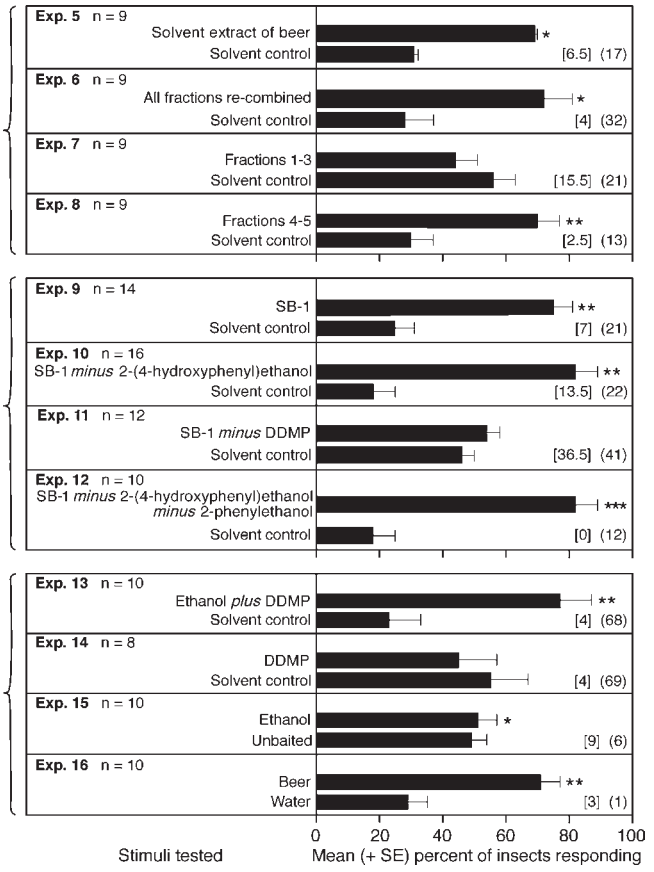

Figure 4. Percentage of male *Blattella germanica* responding in two-choice arena olfactometer experiments 5−16 (Table 1) to fractions (eluted from silica) of solvent-extracted beer (experiments 5−8), a synthetic blend (SB-1) of the four antennal stimulatory compounds in polar fractions 4 and 5 [2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, ethanol, DDMP] (experiment 9), SB-1 lacking one or more of the four components (experiments 10−12), one- or two-component blends of SB-1 (experiments 13−15), or beer itself (experiment 16). All other details were as given in the caption of Figure 1.

DDMP (experiment 23), the two-component blend of ethanol and DDMP (experiment 24), and 1-hexanol (experiment 25) or ethanol (experiment 26) as single components all significantly attracted female GCRs.

DISCUSSION

Our data indicate that the peanut butter-derived semiochemical 1-hexanol and the beer-derived semiochemicals ethanol and DDMP mediate, in part, attraction of male GCRs to these food sources. All compounds also are associated with various types of other food materials and appear to be exploited as foraging cues by the omnivorous GCR.

The nutty flavor and aroma of peanut butter are due to pyrazines that form in the Maillard reaction[31] when peanuts are roasted. The identification of these nutty flavor and aroma volatiles was greatly facilitated through previous characterization of these types of chemicals.[28,29] However, none of these nutty flavor compounds contributed to the attractiveness of peanut butter to GCRs. On the contrary, 1-hexanol, which carries a slightly metallic or fruity aroma, was the only semiochemical detected in our study.

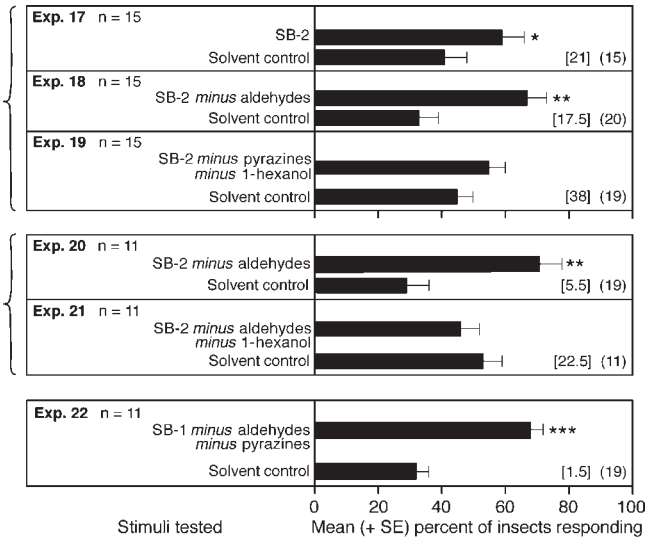

Figure 5. Percentage of male *Blattella germanica* responding in two-choice arena olfactometer experiments 17−22 (Table 1) to a synthetic blend (SB-2) comprising all antennal stimulatory compounds in headspace volatiles of peanut butter (1-hexanol, hexanal, heptanal, nonanal, 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, and 2-ethyl-3,5-dimethylpyrazine) (experiment 17) and to SB-2 lacking one or more groups of the headspace volatiles (experiments 18−22). All other details were as given in the caption of Figure 1.

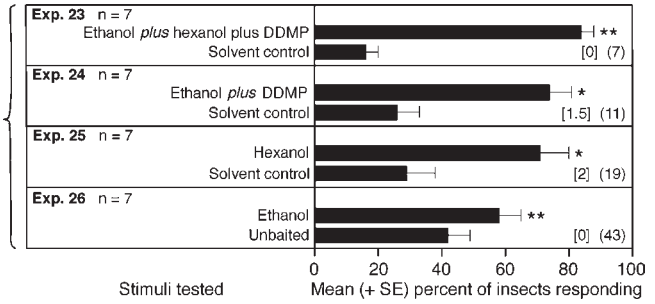

Figure 6. Percentage of male *Blattella germanica* responding in two-choice arena olfactometer experiments 23−26 (Table 1) to three-, two- or one-component blends of beer and peanut semiochemicals. All other details were as given in the caption of Figure 1.

1-Hexanol, together with hexanal, nonanal, 1-octen-3-ol, and (2*E*,4*Z*)-decadienal, is a well-recognized indicator of lipid oxidation and decomposition through the lipoxygenase pathway.[32] It has been reported in headspace volatiles of plant (see, e.g., ref 33) and meat (see, e.g., ref 34) products. Being attracted to 1-hexanol, GCRs may not respond to a compound characteristic of peanut butter. Instead, they may respond to a general indicator of lipids, or lipid decomposition, in diverse food sources of plant or animal origin. If so, this type of response would be comparable to that found in some *Drosophila* fruit flies that forage for and oviposit on a wide range of rotting fruits and vegetable.[35]

Whether 1-hexanol is the only semiochemical in the peanut butter attractive to GCRs is not entirely clear. Neither Porapak Q headspace volatile extract of peanut butter nor synthetic 1-hexanol appeared to be quite as effective as peanut butter in attracting male GCRs[20] (Figures 1, 5, and 6). This, however, could have been due to peanut butter being a better and continuous dispenser of 1-hexanol than cotton was for the release of headspace volatile extract or synthetic 1-hexanol.

The strong semiochemical activity of ethanol and DDMP in beer is based on their synergistic interaction, as DDMP alone fails to attract GCRs. DDMP forms as one of many aromas produced in the Maillard reaction, a chemical reaction between an amino acid and a reducing sugar usually requiring heat. Related 2, 3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one (dihydromaltol) is present in many heated and stored foods and alcoholic beverages (see, e.g., ref 36). It also has been discovered as a food flavor in barley malt (caramalt),[37] a constituent in beer brewing. The caramel-type flavor of both dihydromaltol and DDMP resembles and likely contributes to the smell of stale beer. The potency of the smell to the human nose is surprising, considering that DDMP was present in quantities insufficient for detection in headspace volatiles and barely detectable in solvent extracts of beer.

With the identification of ethanol and DDMP as key semiochemicals in "Pale Ale" beer, it made sense that fresh beer (<12 h after opening the can) and beer aged for 6 days at 6 or 20 °C were equally effective (at a 4 mL dose) in attracting female GCRs (data not shown). The compounds become part of the bouquet through the caramalt brewing ingredient (dihydromaltol) and/or materialize in the brewing process (DDMP, and ethanol).

There was no obvious synergistic or additive effect between peanut butter and beer semiochemicals. Assuming that 1-hexanol stands for lipids (see above) and DDMP for sugars, lipids and sugars were not more appealing than either nutrient type alone. This result is surprising, a more diverse diet having been expected to be more appealing to GCRs than a single food type. A possible explanation is that bioassay insects were maintained on a "balanced" diet and did not have much to gain by selecting a more balanced food source at the time of the bioassay.

In conclusion, our study has revealed key semiochemicals in peanut butter and beer that attract female GCRs. These compounds may be indicative of different nutrient types in diverse food sources of plant and animal origin, thus appealing to the omnivorous taste of GCRs. Synthetic equivalents of these semiochemicals may be formulated as baits or be added to, and thus enhance the attractiveness of, natural food sources as trap or insecticidal baits.

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