SEMIOCHEMICAL-BASED FOOD-FORAGING IN
GERMAN COCKROACHES, BLATTELLA GERMANICA L.
(DICTYOPTERA: BLATTELLIDAE)

by

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Semiochemical-based food-foraging in German cockroaches, *Blattella germanica* L. (Dictyoptera: Blattellidae)

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Abstract

In two-choice, still-air arena olfactometer experiments, Porapak-Q headspace volatile extract of peanut butter and solvent extract of beer were shown to attract males of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). 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 many types of (heated) food. By responding to these rather general food-derived compounds, the omnivorous German cockroaches appear to exploit semiochemicals that indicate the presence of various food types, such as lipids and carbohydrates.

**Keywords:** German cockroach, beer, peanut butter, foraging, semiochemicals.
~ To my parents ~

~ تقدیم به پدر و مادرم ~
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Chapter 1: General Introduction
1.1. Life history

The German cockroach (GCR), *Blattella germanica* (L.), also referred to as the Russian or Polish cockroach or the Croton-bug (Herrick, 2007), most likely originated from northeastern Africa (Rehn, 1945; Cornwell, 1968) rather than southeast Asia as previously suggested (Princis, 1969). As of today, the GCR is a well-known pest worldwide (Princis, 1969).

Virgin females express sexual receptivity 5-7 days after eclosion (Lee and Wu, 1994). Mated females produce 4-8 egg capsules or oothcae (6.5-8.1 mm × 3.1-3.2 mm), containing up to 40 eggs each (Wheeler, 1889; Cornwell, 1968; Mirzayans, 1986; Hill, 1990). Mated females feed and drink intensely before producing oothecae (Cochran, 1983) likely to provide sufficient water and nutrients for developing oocytes (Lee and Wu, 1994; and references cited therein; Mullins et al., 2002), but eat or drink sparingly with the appearance of the ootheca (Cochran, 1983). Females carry an ootheca for about 15-17 days until the eggs are ready to hatch. This type of “oviposition behaviour” is considered transitional between oviparous and ovoviviparous (Roth, 1989; Nalepa and Bell, 1997; Fan et al., 2002). Nymphs undergo 5-7 instar molts during 38-63 days of development depending on temperature (Cornwell, 1968; Hill, 1990). Under optimal conditions, adults may live for up to 153 days depending on gender (Cornwell, 1968; Hill, 1990), and there may be four generations per year (Becker and Hansen, 1987). Adult longevity, omnivory and prolific reproduction, all contribute to the biological success of GCRs, especially in indoor habitats (Milligan, 1984).
1.2. **Diet and food-foraging behaviour**

Cockroaches are generalist omnivores (Raubenheimer and Jones, 2006). ‘They use olfactory cues to find their food, forage individually, but often converge on the same places’ (Rivault and Cloarec, 1991).

Food is an important extrinsic factor in the regulation of moulting and reproduction of GCRs (Kunkel, 1966), and limits the level of population densities (Rivault, 1989; Rivault and Cloarec, 1991). According to Kunkel (1966), starvation following a moult or parturition delays the initiation of another moulting or reproductive cycle in females, but the initiation of a moulting cycle after a period of starvation requires only a short period (12 h) of food availability. Moreover, the ability to postpone development until adequate food becomes available is advantageous to GCRs, but it contributes to the difficulty managing them.

1.3. **Food-foraging behaviour**

Cockroaches forage primarily at night (Broadbent, 1977), and remain concealed in cracks and crevices during the day, unless they are crowded with all developmental stages co-occurring (Drees and Jackman, 1999). Modeling the foraging behaviour of GCRs in urban environments (Krebs et al., 1978; Kacelnik, 1984; McNair, 1982; Kamil and Roitblat, 1985; Stephans and Krebs, 1986; Rivault and Cloarec, 1991) revealed that individuals take into account interpatch travel and patch residence times. According to Rivault and Cloarec (1991), the exploitation of food patches by cockroaches occurs in a “step-by-step manner”, ‘because the risk of being predated or of being lost in an unknown environment when foraging far from the usual shelter is higher than the risk of being deprived of food’. The exploitation of food is affected by its distance from the
shelter. Cockroaches start foraging at the onset of the scotophase, feeding on nearby food items first. Late comers at such food sources preferred further exploiting them and facing competition to foraging farther away from the shelter. Regardless of the amount of food remaining in the closest food patch, the mean number of cockroaches in it increased continuously, reached a maximum, and decreased rapidly after the food was completely consumed (Rivault and Cloarec, 1991). Only then did cockroaches exploit the next closest patch. The same type of pattern applied to all food sources regardless of their spatial position, suggesting that ‘distance does not influence the dynamics of exploitation of a food item’ (Rivault and Cloarec, 1991).

Rivault and Cloarec (1991) further observed that after a food patch was completely exploited, the mean number of cockroaches peaked in a 20-cm diameter circle around the patch, and then decreased rapidly. Subsequently, a rapid increase and slow decrease occurred in the number of insects in a 60-cm diameter circle around the former food patch. Rivault and Cloarec (1991) conclude that GCRs seem to perceive food from outside the 20-cm circle because they located and headed straight to the food-containing centre. The 20-cm circle appeared to be a transit zone only crossed by GCRs to reach the food source or depart from it.

Kells and Bennett (1998) confirmed the distance-related behavioural pattern, adding that during food-foraging and diet selection GCRs take into consideration their diet history and potential nutritional deficiency. Wolfe et al. (1997), however, claim that cockroaches forage randomly, examine food with their mouthparts and antennae and ingest it only if it contains feeding stimulants¹.

¹ Feeding stimulants are defined as dietary constituents that induce ingestion of food.
The overall activity level of adult GCRs and their tendency to forage is dependent upon gender and reproductive status, with males and ootheca-bearing females being the most and least active foragers, respectively, and virgin and mated females ranking in between (Metzger, 1995). DeMark and Bennett (1995) concur with some of these conclusions but claim that mated, non-gravid females are the most active foragers. Rust and Reierson (2007) also conclude that ootheca-bearing females avoid open spaces. First instar nymphs forage sparingly (Kopanic and Schal, 1999) and rely on coprophagy to survive (Kopanic et al., 2001). The foraging activity of nymphs increases with increasing instars, with 5th and 6th instars being more active than 3rd and 4th instars (Metzger, 1995). Necrophagy and cannibalism (Durier and Rivault, 2000) and the ability to return to a previously investigated food resource (Wolfe et al., 1997) are other important phenomena in the food-foraging behaviour of GCRs. Cannibalism may result from nutritional stress (Kells and Bennet, 1998), and necrophagy is typically avoided when food is present (Tabaru et al., 2003). However, even in the presence of a diverse diet, freshly-moulted nymphs may be preyed upon (Karimifar, personal observations).

1.4. Diet
Cockroaches are omnivorous (Brenner et al., 1991; Cloarec et al., 1992; Raubenheimer and Jones, 2006; Weber, 2007), feeding on diverse types of food, including grease, soap, ink, shoe polish, fingernails, eyelashes, hair (United States Environmental Protection Agency (E.P.A.), 2003; Stauffer, 2007; Pest Control Canada, 2008), paper, cardboard (Brenner et al., 1991; Stauffer, 2007), bookbinding, wallpaper paste, paint (E.P.A., 2003; Pest Control Canada, 2008), and glue (E.P.A., 2003).
Unlike adults, first instar nymphs ingest and closely depend on high intake of protein (Kopanic et al., 2001). Results from chemical analysis of adult faeces shows that the protein percentage of faeces depends on the protein levels of the diet, and that first instar nymphs have a higher survival rate when fed on faeces of adults maintained on a high (50%) rather than a medium (22.5%) or low (5%) protein diet (Kopanic et al., 2001). Moreover, the type of protein directly obtained from food affects nymphal development and reproduction. For example, soybean protein is superior to milk, meat and other plant proteins (Cooper and Schal, 1992). Adults that feed on dried carcasses or faeces are not reproductive and do not live as long as well-nourished conspecifics (Tabaru et al., 2003). Overall, female GCRs process more food than males or nymphs (Gore and Schal, 2004).

1.5. Pest status

Among common pest cockroaches, GCRs have the shortest generation time (Rust and Reierson, 2007). Discounting cannibalism and other population pressures, a single female and her offspring can produce over 30,000 offspring per year (Rust and Reierson, 2007). GCRs spread by commerce and mass migration, and due to their small size might successfully hide in small cracks and crevices (Weber, 2007). As a result, GCRs have become the most common roaches in homes, apartments, restaurants, supermarkets, hospitals, hotels, and other buildings where food is prepared, served, or stored (Lyon, no date). If not controlled, GCR populations can double every two weeks (Koehler, 2006).

GCRs are known to transmit pathogens of human illnesses such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. (Brenner, 1995; Fathpour et al., 2003; Koehler, 2006; Mpuchane et al., 2006; Vahabi et al., 2007), and to be an
intermediate host to a nematode of the genus *Gonglyonema* that might cause cancer (Mirzayans, 1986).

Food-spoiling bacteria, yeast, and mould have been isolated from the frass and external body parts of domestic GCRs (Cloarec et al., 1992; Mpuchane et al., 2006) in addition to bacteria known to be carried in the gut (Cloarec et al., 1992). These microorganisms may contaminate food when GCRs moult, defecate, or excrete partially digested food onto surfaces like food preparation sites (De Jonge, 1993; Mpuchane et al., 2006).

The GCR and American cockroach, *Periplaneta americana*, have been linked to the occurrence of asthma, especially among inner-city children (Rosenstreich et al., 1997). In urban settings, exposure and sensitization to cockroaches are associated with greater symptoms in asthmatic children and emergency hospital visits (Rosenstreich et al., 1997). However, cockroach-induced allergies and asthma are not strictly an inner-city phenomenon. There is a link between housing quality, cockroach infestation and the incidence of allergic diseases, in both the inner city and the suburbs across societies (Rona, 2000).

Asthma and most allergic diseases are due, in part, to sensitization and exposure to allergens (Gore and Schal, 2007). Sensitized individuals are allergic to water extracts of GCR tissues, including the intestinal tract, Malpighian tubes, ovaries, ootheca, exuvia, and faeces (Gore and Schal, 2007; and references cited therein). Eight allergens of the GCR are officially recognized and named as GCR allergens as of today (The International Union of Immunological Societies (I.U.I.S.), 2009).
Suppression of cockroach populations and removal of residual cockroach allergens significantly reduces the exposure of individuals to indoor cockroach allergens, thereby decreasing the incidence of allergies and asthma morbidity (Gore and Schal, 2007). Effective suppression of cockroach populations therefore is needed to alleviate health-related problems.

GCRs affect economies in many ways. They are troublesome pests of restaurants, commercial kitchens, and even households, incurring significant expenses for professional pest control services and products. Based on a 1990-survey by E.P.A., at least 24.5% of all households treat for GCR on a somewhat regular schedule (Brenner, 1995). Thus, consumers in the U.S. spend considerable money to control GCRs (Brenner, 1995). Other costs include damage to electronic equipment, sanitation inspections in the food manufacturing industry, and pharmaceutical prescriptions for asthma- and allergy-related health problems caused by GCRs (Brenner, 1995).

1.6. Control methods
Sanitation and exclusion are crucial tactics to effectively manage populations of cockroaches. Strategically placed, adhesive pads are effective in monitoring the presence and estimating the size of cockroach populations.

For controlling cockroaches, pesticide baits are commonly used in various forms including pastes, gels, granules, and dusts (Rust and Reierson, 2007). They usually contain a pesticide mixed with a food type and vary in effectiveness according to cockroach species (Rust and Reierson, 2007) and method of application (Schal, 1992). However, there are several drawbacks associated with such baits. Feeding habits of cockroaches may limit their exposure to gels and baits. Gels dry up and need to be
reapplied (Rust and Reierson, 2007). Insecticides with long half-lives raise safety and environmental concerns and contribute to difficulties of registering them, and the overall results of controlling GCR with insecticides are often unsatisfactory and limited in time and space (Rivault, 1989). GCRs often learn to avoid insecticide-treated food products and evolve insecticide resistance, rendering most insecticides ineffective for long-term control (Cochran, 1999; Wang et al., 2006; Limoe et al., 2007). There are also a few biological control methods. Bioroach bait stations contain the nematode *Steinernema carpocapsae* that “ambush” insect hosts. Other bait stations contain juvenile hormone (JH) analogues that cause abortion of the ootheca in gravid females. Heating premises to temperatures that are lethal to GCRs can also be an effective control method (Gold, 1995) but it is not applicable to many residential dwellings. Amorphous silica aerogel (Drione®), diatomaceous earth or essential plant oils are repellent to and disperse GCRs (Stauffer, 2007) but do not offer long-term control, unless these tactics are integrated in a “push-pull system” (Nalyanya et al., 2000).

1.7. **Research objectives**

Effective attractants for traps or insecticidal baits are considered key components of successful monitoring or abatement programs of GCR populations (Nalyanya and Schal, 2001). Although pheromone-based baits or bait sprays can be effective (Bell et al., 1984; Liang et al., 1998) they are not implemented widely. Baits are typically effective, but may not work in areas where food sources are available (Rust and Reierson, 2007).

Baits are required that release semiochemicals that are very effective irrespective of the insects’ developmental stage or mating status or the presence of competing attractants, such as favoured types of food. My research objectives are to: 1) determine,
or confirm, natural food types with superior attractiveness to GCRs; 2) test whether headspace volatile extracts or solvent extracts of such food types retain attractiveness; and 3) identify and bioassay the key semiochemicals that mediate attraction of GCRs. Synthetic equivalents of these compounds then could be used as lures on their own or to enhance the attractiveness of trap or insecticidal food baits.

1.8. References


   http://www.allergen.org/Allergen.aspx [accessed March 26, 2009].


Chapter 2  Investigation of types of food or food-derived chemicals as attractants for the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae)
2.1. Abstract

Many types of food or food-derived chemicals attract the German cockroach (GCR), *Blattella germanica* (L.) (Dictyoptera: Blattellidae), an omnivorous urban pest worldwide. In two- or four-choice, still-air arena olfactometer experiments, we tested the response of female GCRs to traps baited with rat chow, peanut butter, beer, a dried vegetable mix, dried mint, dried fenugreek, palmitic acid or oleyl alcohol. Traps baited with any of these baits except the latter three were significantly more effective in capturing GCRs than unbaited control traps. Combining food types in anticipation of enhancing their attractiveness had little or no effect. Peanut butter and beer appear to be the least complex and thus most promising food sources for the identification of semiochemicals that mediate attraction of GCRs.

2.2. Introduction

The German cockroach (GCR), *Blattella germanica* (L.) (Dictyoptera: Blattellidae), has often been considered the most common domestic cockroach pest worldwide (Rehn, 1945). It is very adaptable and capable of infesting almost any microhabitat as long as it contains water as a key resource. Access to food, warmth, safety and darkness are additional requisites in preferred habitats. Generally, GCRs leave their shelter and forage at night. Eating and drinking habits of individuals appear dependent upon their developmental stage (Kunkel 1966), gender and reproductive status (Durbin and Cochran, 1985; Silverman, 1986), activity level (Metzger, 1995), the population density (Rivault and Cloarec, 1991; Metzger, 1995), and proximity to food and water (Silverman, 1986).
Although GCRs are considered omnivores, the nutritional composition of their prior meal may affect their selection for subsequent meals (Kells and Bennett, 1998). This may explain why the list of food types and food-derived semiochemicals for attraction of GCR contains all constituents of a balanced diet, including carbohydrates, proteins, lipids (Kells and Bennett, 1998), fruit and vegetables or various combinations thereof.

Fruit, vegetables and related products consumed by GCRs include apple, pear, pomace, boiled raisins, citrus pulp (Stauffer, 2007), yellow onions (Stapleton and Stapleton, 1994), and potatoes (Brenner and Patterson, 1988; Stauffer, 2007).

GCRs are attracted to soft drink syrups (Lofgren and Burden, 1958; Schal and Hamilton, 1990), corn and maple syrup (Spaulding and Pasarela, 1989; Bruey, 1991a; Brenner et al., 1991; Geary, 1992; Wolfe et al., 1997), honey or molasses (Bare, 1945; Spaulding and Pasarela, 1989; Bruey, 1991b and cited references therein) as well as sugar (Bare, 1945; Lofgren and Burden, 1958; Broadbent, 1977; Schal and Hamilton, 1990; Stapleton and Stapleton, 1994; Stauffer, 2007), or specifically fructose (Silverman and Bieman, 1996), and maltose (Lofgren and Burden, 1958; Broadbent, 1977).

Lipids that attract GCRs include butter (Bare, 1945) and various saturated or unsaturated fatty acids, alone or in combinations (Ong, 1989). Proteins that attract to GCRs are of animal or plant origin and include beef, poultry (liver), fish, insect pupae, soybean or anise extract (Wileyto and Boush, 1983; Wolfe et al., 1997; Stauffer, 2007). Attractive essential oils are those of banana, sweet orange, apple and pineapple (Schal and Hamilton, 1990; and cited references therein).
There is also a plethora of “home recipes” suggested as baits for attracting GCRs in human dwellings. According to these recipes effective baits are white flour (Stauffer, 2007), white bread (Ballard and Gold, 1982; Stauffer, 2007), oatmeal (Spaulding and Pasarela, 1989; Geary, 1992; Wolfe et al., 1997; Stauffer, 2007), cocoa (Stauffer, 2007), rice bran (Doi and Nakagaki, 1987), pre-gelatinized tapioca, wheat starch (Brenner and Burns, 1999), corn oil (Lofgren and Burden, 1958; Wolfe et al., 1997) and various corn products, such as corn meal (Stauffer, 2007) and corn distiller’s dried grains with solubles obtained from non-beverage alcohol production (Brenner et al., 1991; Brenner and Burns, 1999).

In addition to, or in lieu of, food-derived attractants, complete diets have been considered as baits for GCRs. For example, Wolfe et al. (1997) suggest a combination of animal and plant proteins, grain food, one carbohydrate and one lipid as an effective bait that may also contain feeding stimuli. Other constituents of diet/bait recipes include palmitic acid and oleyl alcohol (Wileyto and Boush, 1983), an alcohol extract of fenugreek seed (Wileyto and Boush, 1983), cheese (United States Army Center for Health Promotion and Preventive Medicine (USCHPPM), 2003), or whole sweet milk (Stapleton and Stapleton, 1994).

Conclusions as to what attractant(s) should be considered for incorporation in GCR baits are complicated by contradictory results or recommendations. For example, while Warner and Scheffrahn (2006) recommend glucose as an attractant as well as a dietary constituent, Silverman and Bieman (1996) implicate glucose as a compound that

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1 Bait is defined as a composition which induces insects to make oriented movements towards its source.
2 Attractants are defined as semiochemicals (message bearing chemicals) that induce insects to make oriented movements towards the source.
3 Dietary constituent is defined as a component of the diet.
discourages GCRs from consuming fructose baits. Similarly, while Geary (1992) claims that “the addition of oatmeal increases the attractiveness of the bait to roaches”, Silverman and Bieman (1996) argue that oatmeal is a feeding inhibitor for GCRs. Drawing definitive conclusions is further complicated in that authors deployed different experimental designs or tested different types of behavioural responses, such as attraction or feeding stimulation. A strong attractant may be a weak feeding stimulant or vice versa. Understanding the type of response a semiochemical elicits may determine control tactics. Attractive baits in retaining traps would not need a feeding stimulant but poisonous baits without traps would. Finally, recommendations for GCR baits may differ in their reliability, being based on rigorous scientific testing or just observations of food types consumed by GCRs.

There are some food types, however, that seem to be generally well accepted as effective baits for GCRs. These include pet or dog food (Valles et al., 1996; Broadbent, 1997), stale beer (Wileyto and Boush, 1983; Miller and Koehler, 2003; Stauffer, 2007) and peanut butter (Brenner and Burns, 1999; Nalyanya and Schal, 2001). Prior to identifying the essential semiochemicals in select sources that attract GCRs (objective in Chapter 3), the objective of this chapter was to (re)investigate or compare the relative attractiveness of these sources.

Based on scientific or anecdotal evidence as well as feedback from professional pest managers and scientists familiar with GCRs, the following food sources or chemicals were selected for behavioural experiments with GCRs: beer, peanut butter, pet food, dried vegetables including mint and fenugreek, as well as palmitic acid and oleyl alcohol.
2.3. Material and methods

2.3.1. Experimental insects

A colony of GCRs was established with nymphs and adults obtained from the insectary of SC Johnson & Son (Racine, WI, USA). The colony was supplemented with specimens captured in an apartment building in Vancouver (BC, Canada). Insects were reared in Plexiglas™ 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% r.h, 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 (Canada Safeway Ltd., Burnaby, BC, Canada), apple slices, and water. Females used in experiments were up to four weeks old. Each individual was bioassayed only once and placed in a separate rearing cage after the bioassay.

2.3.2. Test stimuli and general experimental design

Test stimuli generally consisted of 4-g or 4-ml aliquots of select solid or liquid food types (Table 2.1). Plant materials, however, were tested at only 0.5-g aliquots to reduce the intensity of smell, and synthetic oleyl acolhol or palmitic acid singly or in combination were tested as 2% active ingredient in 0.5-ml ethanol, as reported by Wileyto and Boush (1983). When they were tested in combination, the amount of each solution was reduced by 50%.

Solid test stimuli were placed in a Petri dish (5 cm D), whereas the control Petri dish remained empty. Liquid test stimuli (except beer) or equivalent amounts of solvents were pipetted onto braided cotton rolls (8 × 1 cm; Richmond Dental, U.S.A.) inside the
Petri dish. Both treatment and control Petri dishes were covered with mesh that allowed food source-derived volatiles to emanate but prevented access of GCRs to the source.

A treatment or control Petri dish was placed inside an electrical trap modified after Mistal et al. (2000). The trap 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 (1st electrode), while other legs were on the inside wall of the can (2nd electrode), resulting in the completion of a 16-V circuit and electrifying and trapping the GCR. Traps were placed at opposite quadrants of the Plexiglas™ (118 × 39.5 cm) arena 10 cm from the wall. In both two- and four-choice experiments (n = 3-15 each), 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, Ontario, Canada), and left to aerate for > 1 h.

Insects were bioassayed under the same light regime as they were kept in the insectary. Experimental replicates were started at the onset of the scotophase (set to 15:00 h) by placing a paper-lined glass tube (40 × 2 cm) containing 20 (± 1) 2-day starved but water-provisioned female GCRs in the middle of the arena and allowing them to exit the tube and to forage for ~21 h.

2.3.3. Specific experiments
Detailed information about all stimuli tested in experiments 1-14 is provided in Table 2.1. Two-choice experiments 1 and 2 tested the attractiveness of traps baited with palmitic acid or oleyl alcohol as a single test stimulus versus unbaited control traps. Four-choice experiment 3 tested whether synthetic palmitic acid and oleyl alcohol in combination as a
trap bait were more effective than either stimulus alone or no stimulus. Two-choice experiments 4-6 tested whether traps baited with a complex food source, such as rat chow (experiment 4), peanut butter (experiment 5), or beer (experiment 6) were more attractive to GCRs than unbaited control traps. Four-choice experiment 7 tested whether peanut butter and chow in combination are more effective as a trap bait than is either one or neither of the two food sources. Similarly, experiment 8 tested whether peanut butter and beer in combination are more effective as a trap bait than is either one or neither of the two food sources. Concurrent, two-choice experiments 9-11 tested traps baited with various types of plant material, such as a dried vegetable mix (consisting of mint, fenugreek, tarragon and sumac; experiment 9), dried mint (experiment 10) or dried fenugreek (experiment 11) versus unbaited control traps.

Taking results of prior experiments into account, the final set of concurrent, two-choice experiments 12-14 explored whether 1-, 2-, or 3-component baits are more effective, by testing dried mint (experiment 12), beer plus peanut butter (experiment 13) or beer plus peanut butter plus dried mint (experiment 14) versus unbaited control traps. Baits in experiments 12-14 were tested in separate arenas rather than head to head because of concern that three proven effective baits in the same area may saturate the headspace and disorient bioassay insects that move towards test stimuli.

The number of insects responding to treatment or control stimuli in two-choice and four-choice experiments was analyzed with the Pearson Chi Square test (Zar, 1999). This procedure tested the null hypothesis that there is no difference in the mean number of insects responding to treatment or control stimuli. In four-choice experiments, the proportion of insects responding to specific sets of two stimuli was also compared by the
logistic regression modeling procedure (GLM), testing the null hypothesis that there is no difference in mean proportions of insects responding to such stimuli. If the p-value of either test statistic was <0.05, there is a statistically significant difference in the proportion of responding insects. All analyses employed JMP™ 7 software (SAS®, Cary, NC, USA).

2.4. Results
In experiments 1-3, traps baited with palmitic acid, oleyl alcohol or both were as ineffective as unbaited control traps in attracting female GCRs (Figure 2.1). In experiments 4-6, however, significantly more females were captured in traps baited with rat chow (experiment 4), peanut butter (experiment 5), or beer (experiment 6) than in unbaited control traps. In four-choice experiment 7, there was a significant difference between insects responding to treatment or control stimuli ($\chi^2$: 26.2791), but not between proportions of insects responding to 1- or 2-component treatment stimuli [peanut butter vs mix: 0.0123 (GLM); chow vs mix: 0.0695 (GLM)]. In four-choice experiment 8, there was a significant difference between insects responding to treatment stimuli ($\chi^2$: 48.7217), with a significantly greater proportion responding to the combination of beer and peanut than to peanut butter (mix vs peanut butter: 4.4362; GLM), but with equal proportions responding to the combination of beer and peanut butter or to beer (mix vs beer: 3.2866; GLM). In experiments 9-11, significantly more females were captured in baited than in unbaited control traps, when the former were baited with a dried vegetable mix (experiment 9) or dried mint (experiment 10), but not when they were baited with dried fenugreek (experiment 11). In experiments 12-14, significantly more females were captured in traps baited with dried mint (experiment 12), beer and peanut butter.
(experiment 13), or baited with dried mint, beer and peanut butter (experiment 14) than in unbaited control traps.

2.5. Discussion

The presented data support previous reports of various food types as suitable baits for attracting GCRs, including pet food (Valles et al., 1996; Broadbent, 1977), stale beer (Wileyto and Boush, 1983; Miller and Koehler, 2003; Stauffer, 2007), and peanut butter (Brenner and Burns, 1999; Nalyanya and Schal, 2001). They also revealed plant sources as potentially potent baits, such as a dried vegetable mix or dried mint, but fail to confirm dried fenugreek, palmitic acid or oleyl alcohol (Wileyto and Boush, 1983) as effective baits.

Pet food has long been used to laboratory-rear various cockroach species, such as the Madagascan hissing cockroach, *Gromphadorhina portentosa* (Darmo and Ludwig, n.d.), American cockroach, *Periplaneta americana*, and the GCR (Lofgren and Burden, 1958; Cochran, 1983; Durbin and Cochran, 1985; Mullins et al., 2002). While it obviously contains all components of a suitable cockroach diet, it also releases semiochemicals that must be very effective long-range attractants, as follows: to be captured in the pet food-baited can trap (15.8 × 16 cm D:H) in our experiments, bioassay insects needed to climb up the outside of the can and descend on the inside to approach the food at the bottom of the can. As the electrocuting retaining mechanism allowed insects to enter but not exit the trap of their first choice, significantly larger captures in pet food-baited than unbaited traps must have been due to the insects’ perception of food-derived semiochemicals over a range of at least 10 cm, the distance between the electrocuting mechanism and the food source. The same interpretation applies to all food
sources that proved to be effective baits. In retrospect, video-tracking the movement of
bioassay insects would have been valuable to determine the distance at which they
initiate oriented movements toward a trap dependent upon the presence and type of bait.
If such video-recording had revealed orientation towards food sources, such data would
have further supported previous conclusions that GCRs can locate food sources from a
distance (Rivault and Cloarec, 1991).

Although pet food was a very strong attractant for GCRs in experiment 4, there is
reason not to recommend it as a simple trouble free bait. Subsequent batches of the very
same brand failed to attract GCRs. These batches also smelled noticeably different, and
generated different gas chromatographic profiles of headspace volatiles (unpublished
data). Different batches may have lacked essential attractants or may have contained
additional volatiles masking them. Irrespective of the correct explanation, pet food
sources not consistently attractive to GCRs don’t lend themselves readily to an
operational GCR bait or as a source for semiochemical identification.

The insignificant response of GCRs to palmitic acid, oleyl alcohol or both in
experiments 1-3 contrasts previous reports of these compounds as GCR attractants
(Wileyto and Boush, 1983). Wileyto and Boush (1983) dissolved palmitic acid and oleyl
alcohol in an ethanol solution, but appear not to have tested ethanol as the control
stimulus. Ethanol (at 200 µl), however, turned out to be one of two key semiochemicals
of beer that attract GCRs (Chapter 3). Thus, in the Wileyto and Boush experiments, the
attractiveness of palmitic acid or oleyl alcohol could have been due in part or entirely to
ethanol. If so, this may also explain why in our experiments 1-3 the ethanol “control” was
as effective a bait as palmitic acid or oleyl alcohol dissolved in ethanol. To properly
determine whether, and how strongly, palmitic acid and oleyl alcohol attract GCRs, they would have to be tested in a solvent other than ethanol.

There was no synergistic interaction between different sources of GCR attractants. However, there was a minor additive effect in that the combination of peanut butter and beer in experiment 8 was more attractive to GCRs than peanut butter (but not than beer). These results are somewhat surprising, having expected the more diverse diet of combined food sources to be much more appealing than single food sources. One explanation is that bioassay insects were maintained on a “balanced” diet and did not likely have any deficiency of particular nutrient types, such as lipids (peanut butter) or carbohydrates (beer). Thus, in bioassays they might have made “food choices” based on their current “cravings” rather than to obtain a balanced meal.

Taking the results of all experiments into account, and also considering that peanut butter and beer are used or recommended for commercial and domestic GCR control (Miller and Koehler, 2003), I have selected beer and peanut butter for the identification of essential semiochemicals in Chapter 3.

2.6. References


Bare, O. S. (1945) Boric acid as a stomach poison for the German cockroach. J. Econ. Ent. 38: 407.


Darmo, L., Ludwig, F. (n.d.) Madagascan giant hissing roaches. Available from the National Health Museum Web site:


### Table 2.1 Stimuli tested in still-air, arena olfactometer experiments 1–14.

<table>
<thead>
<tr>
<th>Exp</th>
<th>n</th>
<th>Treatment (T) stimulus</th>
<th>Control stimulus</th>
<th>Source/Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>Palmitic acid (2% in 0.5 ml ethanol)</td>
<td>Ethanol (0.5 ml)</td>
<td>Sigma-Aldrich&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Oleyl alcohol (2% in 0.5 ml ethanol)</td>
<td>Ethanol (0.5 ml)</td>
<td>Sigma-Aldrich</td>
</tr>
</tbody>
</table>
| 3   | 3 | **T1**: Palmitic acid (2% in 0.5 ml ethanol)  
 **T2**: Oleyl alcohol (2% in 0.5 ml ethanol)  
 **T3**: Palmitic acid & oleyl alcohol (2% each in 0.5 ml ethanol) | Ethanol (0.5 ml) | Sigma-Aldrich    |
| 4   | 5 | Rat chow (4 g) | No bait | Mazuri Rat Chow<sup>4</sup> |
| 5   | 5 | Peanut butter (4 g) | No Bait | Great Value Peanut Butter<sup>5</sup> |
| 6   | 9 | Beer (4 ml) | Water (4 ml) | Pale Ale<sup>6</sup> |
| 7   | 13 | **T1**: Peanut butter (4 g)  
 **T2**: Chow (4 g)  
 **T3**: Peanut butter (2 g) & Chow (2 g) | No bait | As in Exps. 4, 5 |
| 8   | 10 | **T1**: Beer (4 ml)  
 **T2**: Peanut butter (2 g)  
 **T3**: Beer (2 ml) & peanut butter (2 g) | Water (2 ml) | As in Exps. 5, 6 |
| 9   | 10 | Dried vegetable mix<sup>7</sup> (0.5 g) | No bait | Tiar vegetables<sup>8</sup> |
| 10  | 8 | Dried mint (0.125 g) | No bait | As in Exp. 9 |
| 11  | 9 | Dried fenugreek (0.125 g) | No bait | As in Exp. 9 |
| 12  | 10 | Dried mint (0.125 g) | No bait | As in Exp. 9 |
| 13  | 10 | Beer (1.9 ml) & peanut butter (1.9 g) | Water (1.9 ml) | As in Exp. 9 |
| 14  | 10 | Dried mint (0.1 g) & beer (1.9 ml) & peanut butter (1.9 g) | Water (1.9 ml) | As in Exps. 6,7,10 |

<sup>1</sup>Experiments 9-11 and 12-13 were run concurrently  
<sup>2</sup>n=Number of replicates  
<sup>3</sup>Sigma-Aldrich, Oakville, Ontario L6H 6J8, Canada  
<sup>4</sup>Mazuri Rat and Mouse Diet, PMI Nutrition; Jamiesons Pet Food Distributors, Delta, BC, Canada  
<sup>5</sup>Wal-Mart, Coquitlam, BC, Canada  
<sup>6</sup>Okanagan Spring Pale Ale, Okanagan Spring Brewery, BC, Canada  
<sup>7</sup>The 0.5-g mix consisted of 0.125 g each of mint, fenugreek, tarragon, and powdered sumac (all dried)  
<sup>8</sup>Tiar dried vegetables, Mashhad Roosta Co., Iran; purchased at Delijan Market, Burnaby, BC, Canada
2.8. Figure

Figure 2.1 Proportions of female *Blattella germanica* responding in two- or four-choice experiments 1-14 to different types of food or food-derived chemicals (see Table 2.1). In all experiments, $\chi^2$ or GLM values are reported in brackets, the number in parenthesis indicates the percentage of non-responding insects, and an asterisk (*) indicates a statistically significant preference for particular test stimulus at $\alpha = 0.05$. Experiments grouped by brackets were run concurrently; n = number of replicates.
<table>
<thead>
<tr>
<th>Exp.</th>
<th>n =</th>
<th>Stimuli</th>
<th>Mean (+ SE) percent of insects responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>Palmitic acid</td>
<td>[0.0588] (89)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent control</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Oleyl alcohol</td>
<td>[2.6] (82)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent control</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Palmitic acid</td>
<td>[1.2] (83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oleyl alcohol</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Palmitic acid + oleyl alcohol</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent control</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Rat chow</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unbaited</td>
<td>[72.1] (6)</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Peanut butter</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unbaited</td>
<td>[45.0] (16)</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Beer</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>[18.7] (50)</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>Peanut butter</td>
<td>[26.3]*** (66)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chow</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peanut butter + chow</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unbaited</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>Beer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peanut butter</td>
<td>[4.4]** (19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beer + peanut butter</td>
<td>[48.7]*** (19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>Dried vegetable mix</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unbaited</td>
<td>[22.3] (44)</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>Mint</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unbaited</td>
<td>[15.6] (34)</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>Dried fenugreek</td>
<td>[0.2] (37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unbaited</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>Mint</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unbaited</td>
<td>[13.6] (28)</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>Beer + peanut butter</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>[18.9] (23)</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>Mint + beer + peanut butter</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water</td>
<td>[23.8] (33)</td>
</tr>
</tbody>
</table>
Chapter 3  Do general or specific food semiochemicals attract omnivorous German cockroaches, *Blattella germanica*?¹,²,³

¹ Regine Gries assisted in the analysis and identification of candidate semiochemicals.
² Grigori Khaskin synthesized candidate semiochemicals that could not be purchased.
³ A modified version of this Chapter will be submitted as a manuscript to the Journal of Chemical Ecology.
3.1. Abstract
In two-choice, still-air arena olfactometer experiments, Porapak-Q headspace volatile extracts of peanut butter and solvent extracts of beer attracted males of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). 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(3H)-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 flavour is found in several types of food. By responding to these rather general food-derived compounds, the omnivorous German cockroach appears to exploit semiochemicals that indicate the presence of various food types, such as lipids and carbohydrates.

3.2. Introduction
The German cockroach (GCR), *Blattella germanica* (L.) (Dictyoptera: Blattellidae), is one of the most significant urban and food-associated pests worldwide (Rehn, 1945; Cornwell, 1968; Cochran, 1999). Movement of GCRs between organic waste and food materials allows them to acquire, carry and transfer pathogens of human illnesses (Cochran, 1999), such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. (Brenner, 1995; Fathpour et al., 2003; Zurek and Schal, 2004; Koehler, 2006; Mpuchane et al., 2006; Vahabi et al., 2007). Exposure to cockroach-derived allergenic proteins in homes is associated with allergic disease and asthma, particularly in inner-city children
(Rosenstreich et al., 1997). However, extensive sanitation and cockroach control can greatly reduce cockroach allergens in household dust (Arbes et al., 2005; McConnell et al., 2003).

Effective attractants that lure GCRs to traps and insecticide baits\(^1\) can significantly enhance successful abatement programs (Schal and Hamilton, 1990). Because pheromones are typically strong attractants (Bell et al., 1984; Liang et al., 1998; Nalyanya et al., 2000), studies were undertaken to understand the pheromonal communication system of GCRs. Aggregation behaviour of female, male and nymph GCRs is mediated by both attractant and arrestant components. Sakuma and Fukami (1990) isolated and identified ammonia and 12 amines including 1-dimethylamino-2-methyl-2-propanol from frass-contaminated filter paper that attracted conspecifics. Sakuma and Fukami (1993) 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 non-volatile components 3,11-dimethyl-2-nonacosane, 29-hydroxy-3,11-dimethyl-2-nonacosane, 29-oxo-3,11-dimethyl-2-nonacosane and 3,11-dimethyl-2-heptacosanone on the females’ cuticular surface (Nishida and Fukami, 1983; Schal et al., 1990), and a volatile component (Liang and Schal, 1993) that was only recently identified as gentisyl quinone isovalerate (“Blattellaquinone”; Nojima et al., 2005).

There is also evidence for auditory communication signals and cues. Females and nymphs produce click-type sound pulses of circa 10-msec duration and peak frequencies of 7, 9, 11 and 14 KHz that attract nymphs (Mistal et al., 2000). Moreover, in groups,

\(^1\) Bait is defined as a composition which induces insects to make oriented movements towards its source.
GCRs wing fan and gravid females utilize auditory cues associated with wing fanning behaviour when they decide whether or not to enter a shelter (Wijenberg et al., 2008).

Neither synthetic pheromones nor sound have yet been deployed widely in abatement programs. This may be due to their offensive smell (amines), complex structure (blattelastanosides), 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.

Baits based on peanut butter are widely used by pest management professionals, and stale beer is a well known and highly recommended home recipe bait for GCR control (Wileyto and Boush, 1983; Miller and Koehler, 2003; Stauffer, 2007). In two- and four-choice arena olfactometer experiments (Chapter 2), peanut butter and stale beer were shown to attract female GCRs. If the essential semiochemicals of these food sources were known, they could be used to enhance or replace the attractants, such as peanut butter, currently used in traps.

The objectives of Chapter 3 were to (i) Porapak Q capture or solvent-extract (headspace) semiochemicals of beer and peanut butter; (ii) test the attraction of GCRs to these extracts; and (iii) identify the semiochemicals that mediate attraction.

3.3. Methods and materials

3.3.1. Experimental Insects

A colony of GCRs was established with nymphs and adults obtained from the insectary of SC Johnson & Son (Racine, WI, USA). The colony was supplemented with specimens captured in an apartment building in Vancouver (BC, Canada). Insects were reared in
Plexiglas™ 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% r.h, 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 four weeks old. Each specimen was bioassayed only once and placed in a specific rearing cage after the bioassay.

3.3.2. Acquisition of test stimuli

3.3.2.1. Headspace volatiles from peanut butter and beer
Great Value Peanut Butter (100 g; Wal-Mart, Mississauga, Ontario, Canada) was placed into a glass chamber (15.5 ID × 20 cm) and charcoal-filtered air was drawn at 1L/min for 71 h through the chamber and a glass tubing (15 × 1 cm) filled with Porapak-Q (50-80 mesh, Water Associates Inc., Milford, Massachusetts). 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, B.C., Canada) was similar except that the beer was retained in a 250-mL Erlenmeyer flask and aerated for 24 h.

3.3.2.2. 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 < 1-day-old beer were also extracted in a 10-ml ether/methanol (9/1) mixture. After adding the solvent mixture to the beer, it was gently shaken for 10 s after which the solvent supernatant was withdrawn.
3.3.2. Behavioural 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, U.S.A.) retained in a Petri dish (5 cm D), whereas equivalent amounts of solvent were applied onto a control cotton roll (Table 3.1). 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 after Mistal et al. (2000). 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 (1st electrode), while other legs were on the inside wall of the can (2nd electrode), resulting in the completion of a 16-V circuit and electrifying and trapping the GCR. Traps were placed at opposite quadrants of the Plexiglas™ (118 × 39.5 cm) arena 10 cm from the wall. In each of two-choice experiments 1-5; n = 9-13), treatment and control stimuli were randomly assigned to each position. Experimental replicates were started at the onset of the scotophase (set to 15:00 h) by placing a paper-lined glass tube (40 × 2 cm) containing 15 (± 1) 2-day starved but water-provisioned male GCRs in the middle of the arena and allowing them to exit the tube and to forage for ~21 h. 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, Ontario, Canada), and left to aerate for > 1 h.

Concurrent experiments 1-4 (n = 9-13) tested peanut butter vs a no-bait control (experiment 1), aliquots of Porapak Q peanut butter headspace volatile extract vs a pentane solvent control (experiment 2), beer vs a water control (experiment 3), and
aliquots of Porapak-Q beer headspace volatile extract vs a pentane solvent control (experiment 4; Table 3.1).

Proportions of insects responding (trap-captured) in experiments 1-4, and 5-26 (see below) were analyzed by the Wilcoxon test (Zar, 1999).

3.3.3. 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 3.3.3.2), or silica fractions thereof (see below), were analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn et al., 1975; Gries et al., 2002), 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 ID) coated with DB-5, DB-23 or DB-210 (J&W Scientific, Folsom, CA, USA). 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 (Staddon and Everton, 1980). The tip of the antenna was then removed by spring microscissors (Fine Science Tools Inc., North Vancouver, British Columbia, CA), and the severed end of the antenna placed into the opening of a second (indifferent) electrode.

Compounds that elicited antennal responses were analyzed by: (1) 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) (J&W Scientific); (2) retention index calculations (Van den Dool and Kratz, 1963); and (3) micro-analytical treatments. 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, phenethyl acetate (Adams, 1989), hexanal, heptanal, γ-
onalactone, 1-hexanol, 1-decanol, ethyl decanoate (Jennings and Shibamoto, 1980), 2,3-
dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP) (Kim and Baltes, 1996),
octanoic acid (pherobase.net), 2,5-dimethyl pyrazine, 2-ethyl-5-methyl pyrazine, 2-ethyl-
3,5-dimethyl pyrazine (Tas and Kleipool, 1974; Cross et al., 1979)] and with those of
authentic standards.

To identify 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 (0.5 g) in a glass column (14 × 0.5 cm ID). After pre-rinsing the silica gel
with pentane, the extract was applied and compounds 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.

3.3.4. Synthesis and procurement of authentic standards
Synthetic standards were purchased, or synthesized in the Gries-laboratory at SFU
(Tables 3.2, 3.3). Phenylethyl acetate was produced by acetylation of the corresponding
alcohol; ethyl octanoate and ethyl decanoate were synthesized by esterification of ethanol
and octanoic or decanoic acid, respectively; DDMP was synthesized according to Kim
and Baltes (1996); 5-allyl-2,3-dimethyl pyrazine was synthesized by allylation of 2,3-
dimethyl pyrazine with allylithium (Tas and Kleipool, 1974), the latter being generated
in situ from lithium and allyl-phenyl ether (Aldrich) according to Eisch and Jacobs
(1963).
As indicated, procured or synthesized compounds were purified 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 Nova-Pak C₁₈ column (60 Å, 4μm; 3.9 × 300 nm) eluted with 1 mL/min of 100% acetonitrile.

3.3.5. Testing of candidate semiochemicals

Employing the same general experimental protocol as described above (3.3.2.), concurrent experiments 5-8 (n = 9 each) tested beer extract vs 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 3.1).

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, DDMP) (experiment 9) and blends lacking specific components, such as 2-(4-hydroxyphenyl)ethanol (experiment 10), DDMP (experiment 11), or lacking both 2-(4-hydroxyphenyl)ethanol and 2-phenylethanol (experiment 12). Concurrent experiments 14-16 re-tested the attractive blend of ethanol and DDMP (experiment 13) as positive control, each compound alone (experiments 14, 15), or beer as a second positive control (experiment 17).

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, nonanal), and three pyrazines (2,5-dimethyl; 2-ethyl-5-methyl; 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 re-tested 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.

The last set of experiments 23-26 were designed to explore any interactions between key semiochemicals of beer (ethanol, DDMP) and peanut butter (1-hexanol). Specifically, experiment 23-26 tested a 3-component blend of 1-hexanol, ethanol and DDMP (experiment 23), a 2-component blend of ethanol and DDMP (experiment 24), as well as 1-hexanol (experiment 25) and ethanol (experiment 26) singly.

3.4. Results

3.4.1. 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, whereas Porapak Q extract of beer headspace volatiles (experiment 4) was not attractive (Figure 3.1).

3.4.2. GC-EAD and GC-MS analyses of semiochemical extracts

GC-EAD and GC-MS analyses of Porapak Q extracts of beer headspace volatiles (Figure 3.2, top) revealed two alcohols (2-phenylethanol, 1-decanol), three esters (ethyl octanoate, ethyl decanoate, phenylethyl acetate), one acid (octanoic acid), one aldehyde
(decanal) and one lactone (γ-nonalactone) that elicited responses from male GCR antennae. GC-EAD and GC-MS analyses of solvent extract of beer (Figure 3.2, bottom) revealed three antennal stimulatory compounds (2-phenylethanol, DDMP, 2-(4-hydroxyphenyl)ethanol), which were all present in the polar silica fractions 4 and 5 (see below).

In GC-EAD and GC-MS analyses of peanut butter headspace volatile extracts (Figure 3.3), one alcohol (1-hexanol), three aldehydes (hexanal, heptanal, nonanal) and three pyrazines (2,5-dimethyl; 2-ethyl-5-methyl; 2-ethyl-3,5-dimethyl) elicited responses from male GCR antennae (Table 3.3).

3.4.3. Testing of candidate semiochemicals

In concurrent arena olfactometer experiments 5-8, solvent extract of beer (experiment 5), all recombined silica fractions of solvent-extracted beer (experiment 6) as well as polar fractions 4 and 5 (experiment 8) significantly attracted male GCRs, whereas non-polar fractions 1-3 (experiment 7) were not attractive (Figure 3.4). 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, ethanol) significantly attracted males (Figure 3.4), 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 3.4). In concurrent experiments 13-16, the two-component blend of ethanol and DDMP (experiment 13), ethanol (experiment 15) or beer (experiment 16) significantly attracted males (Figure 3.4), 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 head space volatiles (Table 3.3) (experiment 17), and SB-2 lacking aldehydes (experiment 18) were significantly more effective than a solvent control in attracting male GCRs (Figure 3.5), 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 on its own) was significantly more effective than a solvent control in attracting male GCRs, confirming 1-hexanol as a key semiochemical of peanut butter.

In concurrent experiments 23-26 (Figure 3.6) that explored potential interactions between semiochemicals from peanut butter and beer, the 3-component blend of ethanol, DDMP and ethanol (experiment 23), the 2-component blend of ethanol and DDMP (experiment 24), and 1-hexanol (experiment 25) and ethanol (experiment 26) as a single-component all significantly attracted males GCRs.

3.5. Discussion
The presented 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 these 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 flavour and aroma of peanut butter are the result of pyrazines that form in the Maillard reaction (Shen et al., 2007) when peanuts are roasted. The identification
of these nutty flavour and aroma volatiles in this Chapter was greatly facilitated through previous characterization of these types of chemicals (Tas and Kleipool, 1974; Cross et al., 1979) and an “analysis of flavours and off-flavours in food and beverages using PME” (solid phase micro extractions) (Shirey and Sidisky, 1998). Unexpectedly, none of these nutty flavour compounds contributed to the attractiveness of peanut butter to GCRs. On the contrary, 1-hexanol that carries a slightly metallic (Im et al., 2004) or fruity (Luna et al., 2006) aroma was the only semiochemical detected in my study.

1-Hexanol, together with hexanal, nonanal, 1-octen-3-ol, and (2E,4Z)-decadienal, is a well recognized indicator of lipid oxidation and decomposition through the lipoxygenase pathway (Feussner and Westernack, 2002). It has been reported in headspace volatiles of plant (e.g., Wang et al., 1997; Morales and Aparicio, 1999; Luna et al., 2006) and meat (e.g., Carrapiso et al., 2003; Im et al., 2004; Estévez et al., 2005) products. In the headspace of raw pork kept at room temperature, 1-hexanol was one of the prevailing volatile constituents (Soncin et al., 2007). 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 vegetables (Spieth, 1974; Wertheim et al., 2002). Rather than being attracted just to odorants of specific fruits, fruit flies also respond to indicators of general fruit fermentation, including CO₂ (Faucher et al., 2006; and references cited therein).

Whether 1-hexanol is the only semiochemical in peanut butter attractive to GCRs is not entirely clear. Neither Porapak Q headspace volatile extract of peanut butter nor
synthetic 1-hexanol appeared as effective as peanut butter in attracting male GCRs (see Chapter 2). 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 was based on their synergistic interaction, with DDMP alone failing to attract GCRs. DDMP forms as one of many aromas produced in the Maillard reaction (Reese and Baltes, 1992; Kim and Baltes, 1996), a chemical reaction between an amino acid and a reducing sugar usually requiring heat. Related dihydromaltol (2,3-dihydro-5-hydroxy-6-methyl-4(\(H\))-pyran-4-one) is present in many heated and stored foods (e.g., Tatum et al., 1967; Ledl et al., 1976) and alcoholic beverages (e.g., Cutzach et al., 1999). It also has been discovered as a food flavour in barley malt (caramalt) (Fickert and Schieberle, 1998), a constituent in beer brewing. The caramel-type flavour of both dihydromaltol and DDMP resembles and likely contributes to the smell of stale beer. The potency of the smell is surprising, considering that DDMP was present in quantities insufficient for detection in headspace volatiles and barely detectable in solvent extracts of beer.

Other examples of compounds with caramel-like flavour particularly in processed foods include 3-hydroxy-2-methyl-4(\(H\))-pyran-4-one (“maltol”), 3-hydroxy-2-ethyl-4(\(H\))-pyran-4-one (“ethylmaltol”), 4-hydroxy-2,5-dimethyl-3(2\(H\))-furanone (“furaneol”), 4-hydroxy-5-methyl-3(2\(H\))-furanone (“norfuraneol”), 2-hydroxy-3-methyl-2-cyclopenten-1-one (“maple lactone” or “cyclotene”), and 3,4-dihydroxy-3-hexen-2,5-dione (“acetylformoin”) (Figure 3.7). Maple lactone formulated in tablet form has been used as a bait to trap GCRs in Botswana (Mpuchane et al., 2005), and may still be present in
current commercial baits based on their characteristic smell. The common moiety in all the molecules that convey the characteristic caramel-type flavour (Figure 3.7) is a planar enol-carbonyl substructure in a cyclic dicarbonyl compound, as already postulated by Hodge (1967) some 42 years ago. However, not until Engel et al. (2001) discovered acetylformoin as the first open-chain caramel-like smelling flavour compound did it become clear that a cyclic enoloxo group is not essential for the caramel-like aroma, as described in a systematic study on cyclic enolones (Ohloff, 1981).

Given the attractiveness of cyclotene (Mpuchane et al., 2005), and of DDMP in combination with ethanol (this Chapter), it is very conceivable that one or more of the other compounds depicted in figure 3.7 also are attractive to GCRs. Indeed, it would be intriguing to engineer a caramel flavour compound that is (i) most attractive to GCRs, (ii) obtainable through facile synthesis, and (iii) resistant to chemical degradation.

With the identification of ethanol and DDMP as key semiochemicals in “Pale Ale” beer, it made sense why 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 male 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, ethanol). Considering, however, that the barley malt or malting greatly contributes to the characteristic taste and flavour of each type of beer (Goldammer, 2008), it is conceivable that different “maltols” or “DDMPs” mediate attraction of GCRs to different types of beer.

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 having expected a more diverse diet 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, my study has revealed key semiochemicals in peanut butter and
beer that attract male GCRs. These compounds may be indicative of different nutrient
types in diverse food sources of plant and animal origin, thus appealing to the
omnivorous diet of GCRs. Synthetic equivalents of these compounds may be used as
lures on their own or to enhance the attractiveness of trap or insecticidal food baits.

3.6. References

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### 3.7. Tables

Table 3.1 Stimuli tested in still-air, arena olfactometer experiments 1 – 26.

<table>
<thead>
<tr>
<th>Exp</th>
<th>N</th>
<th>Stimulus 1</th>
<th>Stimuli tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>Peanut better (4 g)</td>
<td>Unbaited</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Porapak Q extract (pentane; 105 µl) of peanut butter volatiles (373 GHE)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Pentane (105 µl)</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>Beer (4 ml)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Water (4 ml)</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>Porapak Q extract (pentane; 22 µl) of beer (24 MLHE)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Pentane (22 µl)</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>Ether/methanol extract (10/1; 4 ml) of beer (4 ml)</td>
<td>Ether/MeOH (10/1; 4 ml)</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>Silica fractions 1-5 in pentane/ether (10 ml) of ether/methanol extract (10/1; 4 ml) of beer (4 ml)</td>
<td>Pentane/ether (4 ml)</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>Silica fractions 1-3 in pentane/ether (6 ml) of ether/methanol extract (10/1; 4 ml) of beer (4 ml)</td>
<td>Pentane/ether (4 ml)</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>Silica fractions 4-5 in pentane/ether (4 ml) of ether/methanol extract (10/1; 4 ml) of beer (4 ml)</td>
<td>Pentane/ether (4 ml)</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>Synthetic blend (SB-1)&lt;sup&gt;7&lt;/sup&gt; in ether (54 µl)</td>
<td>Ether (54 µl)</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>SB-1 minus 2-(4-hydroxyphenyl)ethanol</td>
<td>Ether (54 µl)</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>SB-1 minus DDMP</td>
<td>Ether (54 µl)</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>SB-1 minus 2-phenylethanol minus 2-(4-hydroxyphenyl)ethanol</td>
<td>Ether (54 µl)</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>Ethanol (200 µl) plus DDMP (4 µg) in MeCN (16 µl)</td>
<td>MeCN (16 µl)</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>DDMP (4 µg) in MeCN (16 µl)</td>
<td>MeCN (16 µl)</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>Ethanol (200 µl)</td>
<td>Unbaited</td>
</tr>
<tr>
<td>16</td>
<td>10</td>
<td>Beer (4 ml)</td>
<td>Water (4 ml)</td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>Synthetic blend (SB-2)&lt;sup&gt;8&lt;/sup&gt; in pentane (103 µl)</td>
<td>Pentane (103 µl)</td>
</tr>
<tr>
<td>18</td>
<td>15</td>
<td>SB-2 minus aldehydes</td>
<td>Pentane (103 µl)</td>
</tr>
<tr>
<td>19</td>
<td>15</td>
<td>SB-2 minus pyrazines minus 1-hexanol</td>
<td>Pentane (103 µl)</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>SB-2 minus aldehydes</td>
<td>Pentane (103 µl)</td>
</tr>
<tr>
<td>21</td>
<td>11</td>
<td>SB-2 minus aldehydes minus 1-hexanol</td>
<td>Pentane (103 µl)</td>
</tr>
<tr>
<td>22</td>
<td>6</td>
<td>SB-2 minus aldehydes minus pyrazines</td>
<td>Pentane (103 µl)</td>
</tr>
<tr>
<td>Exp.</td>
<td>n$^2$</td>
<td>Stimulus 1</td>
<td>Stimuli tested</td>
</tr>
<tr>
<td>------</td>
<td>-------</td>
<td>------------</td>
<td>----------------</td>
</tr>
<tr>
<td>23</td>
<td>7</td>
<td>Ethanol (200 µl) plus DDMP (4 µg) plus 1-hexanol (0.6 µg) in MeCN/pentane (16 µl/75 µl)</td>
<td>MeCN/pentane (16 µl/75 µl)</td>
</tr>
<tr>
<td>24</td>
<td>7</td>
<td>Ethanol (200 µl) plus DDMP (4 µg) in MeCN (16 µl)</td>
<td>MeCN (16 µl)</td>
</tr>
<tr>
<td>25</td>
<td>7</td>
<td>1-Hexanol (0.6 µg) in pentane (75 µl)</td>
<td>Pentane (75 µl)</td>
</tr>
<tr>
<td>26</td>
<td>7</td>
<td>Ethanol (200 µl) unbaited</td>
<td>unbaited</td>
</tr>
</tbody>
</table>

1 Experiments 1-2, 3-4, 5-8, 9-12, 13-16, 17-19, 20-21 and 23-26 were run concurrently.
2 n=number of replicates
3 Great Value Peanut Butter, Wal-Mart, Mississauga, Ontario, Canada
4 GHE = Gram-Hour-Equivalent; 1 GHE = amount of volatiles released from 1 g of peanut butter during 1 h
5 Pale Ale (see endnote # 6)
6 MLHE = 1-ml-Hour-Equivalent; 1MLHE = amount of volatiles released from 1 ml of beer (Pale Ale, Okanagan Spring Brewery, B.C., Canada) during 1 h
7 SB-1= Synthetic blend 1 [ethanol (200 µl), 2-phenylethanol (200 µg), 2-(4-hydroxyphenyl)ethanol (40 µg), DDMP (4 µg)]
8 SB-2= Synthetic blend 2 [1-hexanol (0.6 µg), hexanal (0.05 µg), heptanal (0.1 µg), nonanal (0.4 µg), 2,5-dimethyl pyrazine (0.8 µg), 2-ethyl-5-methyl pyrazine (0.7 µg), 2-ethyl-3,5-dimethyl (0.25 µg)]
Table 3.2 List of 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.

<table>
<thead>
<tr>
<th>#</th>
<th>Compound</th>
<th>RI</th>
<th>ng/µl</th>
<th>Source</th>
<th>Supplier</th>
<th>Purity(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-Phenylethanol</td>
<td>1116</td>
<td>186</td>
<td>HS/SE</td>
<td>Fluka</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>DDMP</td>
<td>1145</td>
<td>1</td>
<td>SE</td>
<td>SFU</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Octanoic acid</td>
<td>1168</td>
<td>1</td>
<td>HS</td>
<td>Aldrich</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl octanoate</td>
<td>1196</td>
<td>40</td>
<td>HS</td>
<td>SFU</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>Decanal</td>
<td>1207</td>
<td>3</td>
<td>HS</td>
<td>Aldrich</td>
<td>99</td>
</tr>
<tr>
<td>6</td>
<td>Phenylethyl acetate</td>
<td>1258</td>
<td>54</td>
<td>HS</td>
<td>SFU</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1-Decanol</td>
<td>1275</td>
<td>3</td>
<td>HS</td>
<td>Aldrich</td>
<td>98</td>
</tr>
<tr>
<td>8</td>
<td>γ-Nonalactone</td>
<td>1362</td>
<td>1</td>
<td>HS</td>
<td>Bedoukian</td>
<td>98</td>
</tr>
<tr>
<td>9</td>
<td>Ethyl decanoate</td>
<td>1395</td>
<td>12</td>
<td>HS</td>
<td>SFU</td>
<td></td>
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<tr>
<td>10</td>
<td>4-Hydroxy-2-phenyl-ethanol</td>
<td>1424</td>
<td>10</td>
<td>SE</td>
<td>Aldrich</td>
<td>98</td>
</tr>
</tbody>
</table>

*a* Numbers as in figure 3.2A and B  
*b* RI=Retention Index  
*c* 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one  
*d* Amount (ng) per µl in headspace volatile extract or solvent extract (compounds # 2, 10)  
*e* HS=Headspace; SE=Solvent Extract  
*f* Fluka Chemie, Buchs, Switzerland, 260 CH-9471  
*g* Synthesized in Gries-laboratory according to Kim and Baltes (1996)  
*h* Sigma-Aldrich, Oakville, Ontario L6H 6J8, Canada  
*i* Synthesized in Gries-Laboratory by esterification of octanoic acid with ethanol  
*j* Synthesized in Gries-Laboratory by esterification of 2-phenylethanol with acetic acid  
*k* Bedoukian Research Inc., Danbury, CT 06810, USA  
*l* Synthesized in Gries-Laboratory by esterification of decanoic acid with ethanol
Table 3.3 List of 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.

<table>
<thead>
<tr>
<th>#</th>
<th>RI²</th>
<th>Compound</th>
<th>ng/µl</th>
<th>Supplier</th>
<th>Purity (%)</th>
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<tr>
<td>11</td>
<td>870</td>
<td>Hexanal</td>
<td>0.5</td>
<td>Aldrich³</td>
<td>98</td>
</tr>
<tr>
<td>12</td>
<td>895</td>
<td>1-Hexanol</td>
<td>6</td>
<td>Aldrich³</td>
<td>98</td>
</tr>
<tr>
<td>13</td>
<td>908</td>
<td>Heptanal</td>
<td>1</td>
<td>Aldrich³</td>
<td>95</td>
</tr>
<tr>
<td>14</td>
<td>999</td>
<td>2,5-dimethyl pyrazine</td>
<td>8</td>
<td>Aldrich³</td>
<td>98</td>
</tr>
<tr>
<td>15</td>
<td>1077</td>
<td>2-ethyl-5-methyl pyrazine</td>
<td>7</td>
<td>Penta⁴</td>
<td>99</td>
</tr>
<tr>
<td>16</td>
<td>1105</td>
<td>2-ethyl-3,5-dimethyl pyrazine</td>
<td>2</td>
<td>Arcos⁵</td>
<td>99</td>
</tr>
<tr>
<td>17</td>
<td>1015</td>
<td>Dichlorobenzene</td>
<td>1</td>
<td>Aldrich³</td>
<td>98</td>
</tr>
<tr>
<td>**</td>
<td>1173</td>
<td>Allyl-2,3-dimethyl pyrazine</td>
<td>0.25</td>
<td>SFU⁶</td>
<td>95</td>
</tr>
</tbody>
</table>

¹ Numbers as in figure 3.3
² Retention index (Van den Dool and Kratz, 1963) on a DB-5 column
³ Sigma-Aldrich, Oakville, Ontario L6H 6J8, Canada
⁴ Penta Manufacturing, Livingston, New Jersey 07039, USA
⁵ Arcos Organics, Morris Plains, New Jersey 07950, USA
⁶ Synthesized in Gries-Laboratory
3.8. Figures

**Figure 3.1** Percentage of male *Blattella germanica* responding in two-choice arena olfactometer experiments 1-4 (Table 3.1) to peanut butter, beer or their respective headspace volatile extracts. In each experiment, the Wilcoxon T-value is reported in brackets, the number in parenthesis represents the percentage of non-responding 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.

**Figure 3.2** Representative recordings (N = 3) of flame ionization detector (FID) and electroantennographic detector (EAD: male *Blattella germanica* antenna) 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 3.1. Chromatography: DB-5 column; splitless injection; temperature of injection part and FID: 240°C; temperature program: 50°C (1 min), 10°C min⁻¹ to 280°C.

**Figure 3.3** Representative recording (N = 3) of flame ionization detector (EAD: male *Blattella germanica* antenna) to aliquots of Porapak Q headspace volatile extract of peanut butter. Further information on antennal stimulatory compounds 1-7 is provided in Table 3.3; * = Diclorobenzene; ** = tentatively identified as 5-allyl-2,3-dimethylpyrazine. Chromatography as described in figure caption 2.
Figure 3.4 Percentage of male *Blattella germanica* responding in two-choice arena olfactometer experiments 5-16 (Table 3.1) to silica fractions of solvent-extracted beer (experiments 5-8), a synthetic blend (SB-1) of the four antennal stimulatory compounds in polar silica 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), 1- or 2-component blends of SB-1 (experiments 13-15), or beer itself (experiment 16). In each experiment, the Wilcoxon T-value is reported in brackets, the number in parenthesis represents the percentage of non-responding 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.

Figure 3.5 Percentage of male *Blattella germanica* responding in two-choice arena olfactometer experiments 17-22 (Table 3.1) to a synthetic blend (SB-2) comprising all antennal stimulatory compounds in headspace volatiles of peanut butter (1-hexanol, hexanal, heptanal, nonanal, 2,5-dimethyl pyrazine, 2-ethyl-5-methyl pyrazine, 2-ethyl-3, 5-dimethyl pyrazine) (experiment 17), and to SB-2 lacking one or more groups of the headspace volatiles (experiments 18-22). In each experiment, the Wilcoxon T-value is reported in brackets, the number in parenthesis represents the percentage of non-responding insects, and an asterisk (*) indicates a 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.
Figure 3.6 Percentage of male *Blattella germanica* responding in two-choice arena olfactometer experiments 23-26 (Table 3.1) to 3-, 2- or 1-component blends of beer and peanut semiochemicals. In each experiment, the Wilcoxon T-value is reported in brackets, the number in parenthesis represents the percentage of non-responding insects, and an asterisk (*) indicates a 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.

Figure 3.7 Structures of caramel-like smelling compounds with an enol-carbonyl moiety:

A = maltol; B = ethylmaltol; C = dihydromaltol; D = furaneol; E = norfuraneol; F = maple lactone (cyclotene); G = acetylformoin.; H = 2,3-dihydro-3,5-dihydroxy-6-methyl-4($H$)-pyran-4-one (DDMP).
Exp. 1  n = 9
Peanut butter unbaited

Exp. 2  n = 13
Porapak-Q extract of peanut butter volatiles
Solvent control

Exp. 3  n = 12
Beer
Water

Exp. 4  n = 12
Porapak-Q extract of beer
Solvent control

Stimuli tested  Mean (+ SE) percent of insects responding

Gries_GSRB_Fig1
<table>
<thead>
<tr>
<th>Experiment</th>
<th>n</th>
<th>Stimulus Description</th>
<th>Mean (+ SE) % of Insects Responding</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exp. 5</strong></td>
<td>9</td>
<td>Solvent extract of beer</td>
<td><img src="image" alt="Graph" /> (6.5) (17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent control</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td><strong>Exp. 6</strong></td>
<td>9</td>
<td>All fractions re-combined</td>
<td><img src="image" alt="Graph" /> (4) (32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent control</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td><strong>Exp. 7</strong></td>
<td>9</td>
<td>Fractions 1-3</td>
<td><img src="image" alt="Graph" /> (15.5) (21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent control</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td><strong>Exp. 8</strong></td>
<td>9</td>
<td>Fractions 4-5</td>
<td><img src="image" alt="Graph" /> (2.5) (13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent control</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td><strong>Exp. 9</strong></td>
<td>14</td>
<td>SB-1</td>
<td><img src="image" alt="Graph" /> (7) (21)</td>
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<tr>
<td></td>
<td></td>
<td>Solvent control</td>
<td><img src="image" alt="Graph" /></td>
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<tr>
<td><strong>Exp. 10</strong></td>
<td>16</td>
<td>SB-1 minus 2-(4-hydroxyphenyl)ethanol</td>
<td><img src="image" alt="Graph" /> (13.5) (22)</td>
</tr>
<tr>
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<td></td>
<td>Solvent control</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td><strong>Exp. 11</strong></td>
<td>12</td>
<td>SB-1 minus DDMP</td>
<td><img src="image" alt="Graph" /> (36.5) (41)</td>
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<tr>
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<td></td>
<td>Solvent control</td>
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<tr>
<td><strong>Exp. 12</strong></td>
<td>10</td>
<td>SB-1 minus DDMP minus 2-phenylethanol</td>
<td><img src="image" alt="Graph" /> (0) (12)</td>
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<tr>
<td><strong>Exp. 13</strong></td>
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<td>Ethanol plus DDMP</td>
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<td><img src="image" alt="Graph" /></td>
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<tr>
<td><strong>Exp. 15</strong></td>
<td>10</td>
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<td><img src="image" alt="Graph" /> (9) (6)</td>
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<td><strong>Exp. 16</strong></td>
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<td>Beer</td>
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<tr>
<td></td>
<td></td>
<td>Water</td>
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Stimuli tested: Means and standard errors are shown for the percent of insects responding to each stimulus. Significant differences are indicated by asterisks: *p < 0.05, **p < 0.01, ***p < 0.001.
<table>
<thead>
<tr>
<th>Experiment</th>
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<td>SB-2</td>
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<td></td>
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<tr>
<td>Exp. 18</td>
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<td>SB-2 minus aldehydes</td>
<td>** [17.5] (20)</td>
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<td></td>
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<td>SB-2 minus pyrazines</td>
<td>[38] (19)</td>
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<tr>
<td></td>
<td></td>
<td>minus 1-hexanol</td>
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<td>Solvent control</td>
<td></td>
</tr>
<tr>
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<td>11</td>
<td>SB-2 minus aldehydes</td>
<td>** [5.5] (19)</td>
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<td>[22.5] (11)</td>
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<td></td>
<td></td>
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<td>Exp. 22</td>
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<td>SB-1 minus aldehydes</td>
<td>*** [1.5] (19)</td>
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<td></td>
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<td>minus pyrazines</td>
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<td>Solvent control</td>
<td></td>
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<tr>
<td>Exp. 23</td>
<td>n = 7</td>
<td>Ethanol plus hexanol plus DDMP</td>
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Stimuli tested

Gries_GSRB_Fig6
Chapter 4  Concluding Summary
German cockroaches (GCR) remain a significant pest of indoor environments. Development of attractive lures is one key tactic in IPM programs for control of cockroach populations. Hence, I investigated various types of food that attract GCRs, and for selected types that proved very attractive, I identified the essential semiochemicals that attract GCRs. Based on laboratory analyses and behavioural bioassays, the following conclusions can be drawn:

1) Binary choice still-air olfactometer experiments confirmed that beer and peanut butter strongly attract GCRs.

2) Further experimentation showed that GCRs are attracted to headspace volatile extract of peanut butter and to solvent extract of beer.

3) 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.

4) 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.

5) 1-Hexanol is a well known headspace volatile of decomposing lipids, ethanol conveys food fermentation, and DDMP with a caramel-type flavour is found in
several types of food. By responding to these rather general food-derived compounds, the omnivorous German cockroach appears to exploit semiochemicals that indicate the presence of various food types, such as lipids and carbohydrates.

6) Other examples of compounds with caramel-like flavour particularly in processed foods include 3-hydroxy-2-methyl-4(\(H\))-pyran-4-one ("maltol"), 3-hydroxy-2-ethyl-4(\(H\))-pyran-4-one ("ethylmaltol"), 4-hydroxy-2,5-dimethyl-3(2\(H\))-furanone ("furaneol"), 4-hydroxy-5-methyl-3(2\(H\))-furanone ("norfuraneol"), 2-hydroxy-3-methyl-2-cyclopenten-1-one ("maple lactone" or "cyclotene"), and 3,4-dihydroxy-3-hexen-2,5-dione ("acetylformoin")

7) The common moiety in all the molecules listed under point 6 that convey the characteristic caramel-type flavour is a enol-carbonyl substructure.

8) Given the attractiveness of cyclotene, and of DDMP in combination with ethanol, it is conceivable that other compounds with an enol-carbonyl substructure are attractive to GCRs.

9) Future research may attempt to engineer a caramel flavour compound that is (i) most attractive to GCRs, (ii) obtainable through facile synthesis, and (iii) resistant to chemical degradation.