New Food Baits for Trapping German Cockroaches *Blattella germanica* (L.)

by

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Abstract

New trap baits were designed and tested for attracting German cockroaches (GCRs), Blattella germanica. In large-arena laboratory experiments, traps baited with rye bread captured 8-fold more GCR males than unbaited control traps. Neither beer nor water enhanced the attractiveness of bread. As Porapak Q headspace volatile extracts of rye bread attracted GCRs, all odorants in extracts were identified by gas chromatography-mass spectrometry. A blend of synthetic rye bread odorants and other known bread odorants was highly attractive to GCRs but the essential components in that blend are yet to be determined. In and field trapping experiments, both a 3-component composition (3CC) [dry malt extract (DME), water, Brewer's yeast] and DME alone were as effective for attracting GCRs as a commercial cockroach bait. Future studies will investigate lethal biocontrol agents that can be added to the 3CC, or the DME, and will explore the efficacy of such baits for GCR control.

Keywords: *Blattella germanica*; attraction; beer; bread; Brewer's yeast; commercial cockroach bait; synthetic bread odorants

Dedication

This thesis is dedicated to my mother and father, Dorothy and William Pol, I would not have made it this far without their love, support and encouragement. To my sister Sarah, who pushes me to be the best I can and has unknowingly been a role model for academic achievement. To my grandfather, Kees VanKasteren, who has always encouraged me to do what I love. To Luiza Magalhães F. Gomes for her love, reassurance, guidance and humour during the final stretch.

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List of Acronyms

3CC	Three-component composition (DME, water and Brewer's yeast)
BY	Brewer's Yeast
CLR	Combat® Source Kill Max Large Roach Combat
CRG	Combat® Source Kill Max Roach Gel
CSR	Combat® Source Kill Max Small Roach Bait
DDMP	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
DME	malted barley powder or dry malt extract
GCR(s)	German Cockroache(s) (Blattella germanica L.)
GHE	Gram hour equivalents
Logit	Log odds ratio of events
MB	Master Blend
RH	Relative Humidity

Glossary

Attract	To be motivated to physically approach something
Attractant	Something that causes an organism to be motivated to physically approach it
Bait	Food used to entice an organism to approach and feed
Foraging	Searching for food
Lure	Something that causes an organism to be motivated to physically approach it
Semiochemical	Message-bearing chemical
Synthetic	Made by chemical synthesis, particularly to imitate a natural product
Trap	A device or enclosure designed to catch an organism or deliver a toxicant

Chapter 1.

Introduction

1.1. General background, taxonomic placement, and geographic distribution

Cockroaches are among the most notorious insects. Fossil records of remarkably diverse roach-like insects date back 360 - 280 million years to the Upper Carboniferous period sometimes referred to as “The Age of Cockroaches” (Marshall 2006; Mallis 2011;Bai *et al.* 2016;), attesting to the resilience and adaptability of these insects in the face of changing environments (Marshall 2006).

Cockroaches are members of the Order Blattaria (Blattetidae) within Dictyoptera (cockroaches, termite and mantids). Cockroaches are separated into six families Cryptocercidae, Polyphagidae, Blattidae, Nocticolidae, Blattellidae and Blaberidae. (Triplehorn and Johnson 2005). All cockroaches are hemimetabolous, meaning that they undergo gradual metamorphosis characterized by three distinct stages – egg, nymph and adult. Currently, there are over 4,500 identified species of cockroaches, in the evolutionarily advanced families of Blattellidae and Blaberidae, with new species being discovered on a regular basis (Mallis 2011). Likely another 5,000 + species are still to be discovered according to experts (Triplehorn and Johnson 2005; Marshall 2006; Mallis 2011;).

Although typically considered cosmopolitan (distributed wherever humans live, from above the Artic Circle to structures in remote deserts), most cockroaches are found in the warm and humid habitats of tropical rainforests and are ubiquitous in almost all habitat types where insects occur (Marshall 2006; Mallis 2011). Cockroaches that are ≤ 12 mm in size are taxonomically placed into the family Blattellidae which comprises many major pest species (Milligan 1984).

A contributing factor to the outstanding survival* of cockroaches is their ability to live on a great number of food types and their tolerance to low temperatures.

*Outstanding survival does not refer here to their supposed ability to withstand radiation, drowning or starvation as the popular media might suggest (Mallis 2011).

Currently, there are 67 species of cockroaches known in North America (Pratt 1988; Atkinson et al. 1991) 24 of which are considered exotic, introduced through human travel and trade. Not all of them are pests; some contribute to the natural decomposition of forest litter, plant materials, woody chips, leaf chips, arthropod cuticle, fungi and algae (Schal et al. 1984; Brenner et al. 1988; Marshall 2006). The origin of most common domestic species (genus *Blattella*) of cockroaches, including German cockroaches, has been contested by several experts. Rehn (1945) suggests German cockroaches originated in the cradle of life, the equatorial region of East Africa. However, because of the great diversity of the genus *Blattella* in Southeast Asia others argue that this was where German cockroaches speciated and from which they migrated (Rhen 1945; Cornwell 1968; Marshall 2006; Mallis 2011;). Nonetheless during the Carboniferous period these two continents would have been in contact in the supercontinent Gondwana and inter-continental movement would not have been an issue.

Cockroaches are thought to have begun their journey with humans when we were cave dwellers, (Roth and Willis 1960). The presumed dispersal patterns of German cockroaches (GCR), *Blattella germanica* L., is described by Cornwell (1968). It is believed that several centuries ago GCRs found their way into eastern Europe on Greek and Phoenician ships, thus spreading to Byzantium and Asia Minor, the Black Sea. From Russia GCRs spread westward and northward across Europe and into America (Schweid 1999). The slow rate of spread into Europe is attributed to the slow development of trade with Russia (Rehn 1945). Once in Western Europe, GCRs quickly spread to virtually all parts of the world, primarily by trade.

GCRs became established in England in the middle of the last century, where they supposedly established in Leeds by means of bread baskets carried by soldiers returning from the Crimean War (Cornwell 1968). Introduced through trade with Europe, GCRs are now common household pests in the US, being most abundant in the warmest States (Cornwell 1968).

There are four main pest species of cockroaches in Canada and the US (Triplehorn and Johnson 2005), the American cockroach, *Periplanetia americana*,

Oriental cockroach, *Blatta orientalis*, brown-banded cockroach, *Supella longipalpa*, and smoky brown cockroach, *Periplaneta fulginosa* (Bell et al. 2007). Ironically, the common names of these cockroaches do not refer to their original distribution (Marshall 2006) but were assigned by humans blaming neighbours for pest problems. Other common names for German cockroaches are Croton bugs (named after a mass migration event in New York; see Howard 1895) as well as shiner and steam flies (Rehn 1945). These 4 species are not native to North America, but originated in the tropics and were inadvertently introduced to the rest of the world via commercial transport and trade, causing ≥1 billion dollars annually for control measures on hotels, restaurants, boats, aircrafts and in households (Marshall 2006).

The focal species of my thesis is the GCR, one of the most widespread and common cockroaches in urban settings (Schal et al. 1984). GCRs account for most cockroach infestations in human dwellings in the US (Mampe, 1972).

1.2. Biology of GCRs

GCRs are among the most well known of the fifty-one-species found in *Blattella* (Roth 1985, 1995). They are the smallest of all the domestic cockroaches, with adults typically reaching only 10-15 mm in length (Bell *et al.*, 2007). Adults are easily identified by their light yellow- to brown-coloured wings and two distinctive black longitudinal stripes on their pronotum (back). The male is light brown with a slender abdomen. Females are typically darker and have a broader and rounded abdomen. Nymphs, in contrast, also have longitudinal streaks that extend down the back but are smaller and lack fully developed wings (Capinera, 2010; Marshall, 2006; Milligan, 1984).

Females may carry a pearly, grain-shaped oothecal egg casing (Marshall, 2006; Milligan, 1984). Sex determination of nymphal GCRs is nearly impossible. The lifespan of GCRs varies according to food availability and ambient temperature. In the laboratory, adult males have lived to survive for 128 days and adult females for 153 days (Ogata 1976; Willis, Riser, and Roth 1958).

1.2.1. Development

GCRs are hemimetabolous with three distinct stages: egg, nymph and adult. The developmental time is dependent on the temperature (30 ºC optimal), the amount and quality of available food (Milner and Pereira 2007) the sex and the social environment (Mallis 2011). Not surprisingly, GCRs are found primarily in restaurants, cafes and large buildings which are typically heated for long periods of time in the winter (Tsuji and Mizuno 1973). Exposure to 5.5 ºC for ≥ 10 days arrested hatching of eggs and exposure to the same temperature for 3 days killed first instar nymphs and 20 days killed adults (Tsuji and Mizuno 1973).

1.2.2. Ootheca

The ootheca is typically oblong-shaped (3 mm wide × 8 mm long) and light to tan in colour and may contain 3-50 eggs (Cornwell 1968; Hill 2002; Willis *et al.* 1958). The ootheca is carried by a female for about 17 - 21 days just until the eggs hatch (Mallis 2011). a unique trait of GCRs (Schal *et al.* 1984). Occasionally, an ootheca is dropped prematurely due to insecticidal applications or other stimuli, thus killing the eggs (Parker and Campbell 1940).

1.2.3. Nymphs

Nymphs of both males and females develop through six instars with up to 63 days, dependent primarily on temperature, the amount and quality of food and water available, sex and the social environment (Cornwell 1968; Hill 2002; Mallis 2011; Willis *et al.* 1958). When first-instar nymphs emerge from the ootheca, they are white but darken within a few hours. Although they are just a few millimeters long, they can climb vertical glass (Willis *et al.* 1958). First and second instars are 3 – 5 mm in size, completely black, and look almost beetle-like (Ebeling 1975). As they develop and molt, they increase in size, broaden, lighten in colour, and lengthen their antennae (Ebeling 1975). Specific instar designations can be assigned based on the number of cerci segments and pronotal width (Tanaka and Hasegawa 1979). Nymphs suffer high levels of mortality due to their sensitivity to desiccation (Milligan 1984).

1.2.4. Adults

Adults are winged and their integument is light-coloured. The wings are thin and membranous, and capable of sustained flight, even though GCRs more commonly run rather than fly (Milligan 1984).

Five to seven days after their molt to adults, GCRs become sexually receptive. Females may produce 4 –8 oothecae during their lifetime, with each ootheca containing about 40 eggs, typically more eggs when females are younger (Cornwell 1968; Hill 2002; Lee and Wu 1994; Mallis 2011). If a female drops her ootheca prematurely, she develops a new one within a few days, more quickly than during typical incubation periods (Willis *et al.* 1958). In laboratory colonies, females may reproduce continuously depositing an ootheca every 17-34 days (Willis *et al.* 1958), but in field populations may remain reproductively inactive for periods of time (Keil 1981).

During courting, GCR males raise their wings, exposing a dorsal gland which produces a nuptial gift. When a female mounts the courting male and feeds on his gland secretion, he clasps her genitalia and initiates copulation (Cornwell 1968; Roth and Willis 1952; Willis *et al.* 1958).

1.3. Communication ecology and social biology of GCRs

GCRs have a primitive social order (referring to their social nature but lack of reproductive labour division and cooperative care of young (Costa 2006) and social nature relating only to their courting behaviour, aggregation and kin recognition) (Lihoreau, Costa, and Rivault 2012) and tend to live in groups (Lihoreau *et al.* 2012). Nonetheless, it appears that GCRs are cooperative, exhibiting such behaviour as aggregation and feeding that require interactions between individuals (Lihoreau, Costa, and Rivault 2012). Cooperative behavior facilitates group cohesion, affords group benefits (e.g., thermal gain, stable hygrometry, reduced predation risks), and allows for rapid information transfer via communication signals. Dispersal, aggregation, mate attraction, mating, development, and kin recognition are all mediated by pheromones (Eliyahu, Nojima, Mori, and Schal 2009).

1.3.1. Aggregation

The aggregation pheromone is a blend of volatile components that mediate long-distance communication. This pheromone emanates from the feces of juvenile and adult GCRs in and around shelters and appears to consist of attractant and arresting components (Mallis 2011). The pheromone induces GCR aggregations that provide safety in numbers (Lihoreau & Rivault 2011), facilitate mate location (Ishii and Kuwahara, 1968; Sakuma and Fukami 1990) and hasten the development of nymphs (Shoziro Ishii and Kuwahara, 1967).

Aggregation behavior by GCRs was first observed by Ledoux (1945) who predicted that chemicals play a role. Rust and Appel (1985) revealed that aggregating GCRs respond to GCR feces, suggesting that the pheromone was feces-derived. Further studies supported this interpretation and also showed that feces arrested first instar nymphs of both GCRs and *P. americana* (Bell, Parsons, and Martinko 1972; Burk and Bell 1973; Ishii 1970; Ishii and Kuwahara 1967). Follow-up experiments aimed at deciphering the pheromone that was first thought to be produced by rectal pads (Ishii and Kuwahara 1967). The pheromone comprises non-volatile arrestment-causing components [Blattellastanoside A and B (Sakuma and Fukami 1993)] and volatile components, including carboxylic acids (Fuchs, Franke, and Francke 1985; Scherkenbeck *et al.* 1999) ammonia, alkyl amines, amino alcohols, and alcohols (Sakuma, Fukami, and Kuwahara 1997). Most recently, carboxylic acids that are part of the aggregation pheromones have been linked to gut microbes of GCRs (Wada-Katsumata *et al.* 2015).

1.3.2. Dispersal

In response to crowding in shelters, juvenile and adult GCRs produce non-volatile salivary gland secretions that induce dispersal (Fauldel, Fuchs, and Nagl 1989; Ross and Tignor 1985, 1986; Suto and Kumada 1981; Tignor and Ross 1987). Several hypotheses have been put forth to explain the need for a dispersal pheromone (Fauldel *et al.* 1989; Ross and Tignor 1985, 1986; Suto and Kumada 1981; Tignor and Ross 1987). The dispersal pheromone may help prevent crowding (Suto and Kumada 1981) and protect early instar nymphs from cannibalism (Ross and Tignor 1985, 1986). More recently, acoustic signals have been attributed to the dispersal or non-aggregation of

individuals to a group (Wijnberg *et al.* 2008). Irrespective, information transfer through these non-volatile salivary gland secretions seems limited and the ensuing dispersal response is slow (Faulde, Fuchs, and Nagl 1990).

1.3.3. Mate attraction

Several sex attractant and mate recognition pheromones are known for GCRs (Liang and Schal 1993; Gemeno and Schal 2004; Nojima *et al.* 2005). Females engage in active “calling” behaviour by stilting their body above the substrate, tilting their thorax down, raising their tegmina and flight wings (Liang and Schal, 1993) and releasing the sex attractant pheromone blattellaquinone (Nojima *et al.* 2005).

1.4. Foraging behaviour

GCRs are primarily nocturnal. Peak periods of feeding and drinking coincide with nocturnal activity peaks (Hocking 1958; Dreisig and Nelson 1971). If the harborage is near food and water resources, some individuals may forage without distinct activity periods (Silverman 1986). Food resources distantly located from a harbourage are less frequently visited (Silverman 1986), possibly because they are harder to find.

1.5. Diet

Food is an important extrinsic factor affecting growth, development and moulting events of GCRs (Kunkel 1966). Food preferences of GCRs vary in accordance with their reproductive stage (Dabouineau and Rivault 1988; Kunkel 1966; Mueller 1978) and the composition of their prior meal(s) (Cloarec *et al.* 1992; Kells and Bennet 1998; Raubenheimer and Jones 2006; Lafferty and Kuris 2009). Starvation can delay or inhibit copulation and prompt oocyte resorption (Kunkel 1966).

Diet composition affects the physiology and behaviour of GCRs. Low protein content in the diet reduces the hatching success of eggs, and prompts females to elevate consumption rates but has no effect on sexual maturation of males (Hamilton and Schal 1988).

1.6. Pest status

GCRs are among the most significant urban and food-associated pests worldwide. GCRs are often found residing on, and moving between, waste organics and human food. In the process, they may acquire, carry and transfer to humans bacteria (Bennet 1993; Cotton *et al.* 2000; Devi and Murray 1991; Gliniewicz *et al.* 2003; Kim, Jeon, and Lee 1995; Kopanic, Sheldon, and Wright 1994; Paul *et al.* 1992; Prado *et al.* 2002; Rivault, Cloarec, and LeGuyader 1993; Roth and Willis 1960; Salehzadeh, Tavacol, and Mahjub 2007; Sramova *et al.* 1992), viruses (Lawson and Johnson 1970; Roth and Willis 1960), fungi (Fotedar and Banerjee 1992; Roth and Willis 1960; Salehzadeh *et al.* 2007) and parasites (Salehzadeh *et al.* 2007). In addition, GCR-derived allergens have been found in infested homes, causing or contributing to allergic diseases and asthma in inner-city children (Do, Zhao, and Gao 2016; Mpuchane *et al.* 2006; Pomés and Arruda 2014; Eggleston *et al.* 1997).

1.7. Research Objectives

Successful cockroach abatement programs are fundamentally constrained by the lack of effective attractants to be deployed in retainer traps or insecticide-laced bait stations. My research objectives (O) in chapter 2 are to: (O1) determine attraction of GCRs to rye bread soaked in beer or water; (O2) assess the contribution of food and water to the attractiveness of the food-and-water bait as a function of GCR water deprivation; (O3) determine the relative attractiveness of bread crust and bread crumbs to GCRs; (O4) identify all headspace odorants (volatiles emitted) of our rye bread bioassayed under O1-O3; and (O5) determine the attractiveness of identified volatiles assembled together in various combinations to produce a bread like bouquet. My research objectives in chapter 3 are to: (O1) bioassay attraction of GCRs to a custom-designed fermenting mixture comprising malted barley, yeast and water; (O2) investigate effects of manufacturing variables (boiling, fermentation time) on the attractiveness of the fermenting mixture; and (O3) compare the attractiveness of the fermenting mixture to that of commercial GCR baits in laboratory and field experiments

1.8. References

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Chapter 2. Rye bread and synthetic bread odorants – effective trap bait and lure for German cockroaches[1]

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2.1. Abstract

Bread-in-beer and bread-in-water are prevalent home recipe trap baits for attracting German cockroaches (GCRs), Blattella germanica L., which are significant urban pests. Our objectives were to (1) test the attractiveness of these baits, (2) study the underlying factors of GCR attraction, and (3) determine if a blend of synthetic bread odorants could replace bread in a trap lure. In large-arena laboratory experiments with laboratory-reared GCR males, traps baited with rye bread not only captured 8-fold more males than unbaited control traps but also most males released into bioassay arenas. Neither beer nor water enhanced the attractiveness of bread. Bread crust as a bait was more effective than bread crumbs. As Porapak Q headspace volatile extracts of rye bread attracted GCRs, all rye bread odorants in extracts were identified by gas chromatography-mass spectrometry. Synthetic rye bread odorants and other known bread odorants were then assembled into a Master Blend. This Master Blend, and even partial blends lacking certain groups of organic volatiles such as aldehydes and ketones, proved very attractive to GCRs. We conclude that rye bread could be used as an effective bait in retainer traps, or, laced with insecticide, as a food source in bait stations. A lure of synthetic bread odorants may eventually replace bread as bait, but the minimum number of essential odorants for that lure has yet to be determined.

Key words: German cockroaches, Blattella germanica, foraging, bread, bread crust, bread crumb, synthetic bread odorants, water, beer, trap bait, trap lure, trapping

2.2. Introduction

German cockroaches (GCRs), Blattella germanica L. (Dictyoptera: Blattetidae), are significant urban pests worldwide. GCRs feed on organic waste, animal feces, kitchen grease and many types of food prepared for human, pet and livestock consumption. In the process, GCRs may acquire, carry and transfer onto human food a cornucopia of bacteria (Roth and Willis 1960; Devi & Murray, 1991; Paul et al., 1992; Sramova et al., 1992; Bennet, 1993; Rivault et al., 1993; Kopanic et al., 1994; Kim et al., 1995; Cotton et al., 2000; Prado et al., 2002; Gliniewicz et al., 2003; Salehzadej et al., 2007), viruses (Roth & Willis, 1960; Lawson & Johnson, 1970), fungi (Roth & Willis, 1960; Fotedar & Banerjee, 1992; Salehzadej et al., 2007) and parasites (Counselman et al., 1989; Marty, 1998; Graczyk et al., 2005; Salehzadej et al., 2007) that cause human illnesses. Moreover, children exposed to GCR-derived allergic proteins suffer from asthma and allergic diseases (Rosenstreich et al., 1997; Mpuchane et al., 2006; Pomes & Arruda 2014; Do et al., 2016). However, proper sanitation and effective GCR control can significantly reduce GCR allergens in household dust.

The success of GCR abatement programs relies, in part, on potent attractants that lure GCRs to traps or insecticide baits (Reierson & Rust, 1977; Schal & Hamilton, 1990; Kells & Bennet, 1998; Buczkowski et al., 2001; Nalyanya & Schal, 2001; Wang & Bennett, 2006). One search for these attractants has focused on pheromonal and sound signals that GCRs use for communication. Aggregation or arrestment pheromones (Ishii & Kuwahara, 1967; Ishii & Kuwahara, 1968; Bell et al., 1972; Sakuma & Fukami, 1990, 1993; Scherkenbeck et al., 1999; Wada-Katsumata et al., 2015), sex attractant pheromone (Liang & Schal, 1993; Nojima et al., 2005) and mate recognition pheromone (Nojima et al., 2005; Lihoreau et al., 2007; Lihoreau et al., 2016) have been identified. Moreover, various sound signals, produced by GCR females and nymphs, attract nymphs (Mistal et al., 2000) and help adult females decide whether to enter a shelter (Wijenberg et al., 2008). However, none of these pheromonal or sound signals has yet been incorporated into commercial trap baits, possibly because they smell bad (amines; aggregation pheromone), attract only prospective mates (sex pheromone) or are technically challenging and expensive to reproduce (sound signals).

Other targets for attractive trap baits are specific types of food (and their odorants) that GCRs prefer. Of the many home recipes used for GCR attraction (Table

1), peanut butter, stale beer and bread are commonly recommended. Water, also, is an essential resource for GCRs and is attractive by itself under certain conditions (Cornwell, 1968; Ross, 1981). GCRs can survive without food for a long time, but not without water (Willis & Lewis, 1957). The importance of access to water may explain why GCRs are often in or near kitchen sinks or bath taps that have residual water or high relative humidity and warmth (Cornwell, 1968; Milligan, 1984; Eggleson & Arruda, 2001; Dingha et al., 2016). Because GCRs rely on food and on water for survival, trap baits that contain a combination of food and water may be more attractive than trap baits limited to food or water.

The key semiochemicals (message bearing chemicals) of stale beer that synergistically attract GCRs are ethanol and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) (Karimifar et al., 2011). DDMP and related odorants form in the brewing process during the Maillard reaction, a chemical reaction between amino acids and reducing sugars, typically requiring heat. As similar types of odorants are produced during the baking process of bread (Cho & Peterson, 2010), particularly in its crust, we studied the attractiveness of bread, and specifically the attractiveness of bread crust.

Commercial food baits require a long and predictable shelf- and field-life. Microbe-exposed natural food baits may deteriorate rapidly and lose their attractiveness to GCRs. Therefore, it is advantageous to identify the key semiochemicals from those food types that strongly attract GCRs. Synthetic semiochemicals that are readily available, stable and inexpensive, may offer a viable alternative to natural food as trap bait.

Our objectives (O) were to (O1) determine attraction of GCRs to rye bread soaked in either beer or water; (O2) assess the contribution of bread and water to the bait attractiveness as a function of GCR water deprivation; (O3) determine the relative attractiveness of bread crust and bread crumbs to GCRs; (O4) identify all headspace odorants in the rye bread bioassayed under O1-O3; and (O5) determine the attractiveness of synthetic bread odorants.

2.3. Materials and Methods

2.3.1. Experimental Insects

A GCR colony was established in 2004 (Karimifar et al., 2011) and maintained in the Insectary Annex of Simon Fraser University, Burnaby, BC, and was supplemented with specimens captured in apartment buildings in Vancouver (British Columbia, Canada) and adjacent municipalities. Cockroaches were kept in Plexiglas® cages (30 cm wide × 60 cm long × 45 cm high) fitted with two mesh-covered openings for air circulation, and maintained at 25 ± 1 °C and 40-70% relative humidity under a photoperiod of L14:D10. Within cages, panels of narrowly spaced particle board and paper towel coverings provided shelters. The diet consisted of Purina® Dog Food (Purina Canada, Mississauga, ON, Canada), fresh apple slices and water.

2.3.2. General Bioassay Procedure

For each experimental replicate, 12 GCR males (total) were collected from rearing cages in two plastic tubes (12.0 cm long × 3.0 cm diameter) containing a paper towel (1.5 cm × 4.0 cm) and a cotton ball with or without water. Prior to bioassay, males were starved for 48 h, unless otherwise stated, to enhance their response to food or food volatiles. Males, instead of females or nymphs, were bioassayed because they are considered the most active foragers (Metzger, 1995). However, we also have data from a parallel food preference study with GCRs (JP, unpubl.) that males, females and mid- to late-instar nymphs respond similarly.

Experiments started at the onset of the scotophase (set to 15:00 h). The two tubes (see above) were placed at the centre of a cylindrical Plexiglas® still-air arena (120 cm diameter × 42 cm high; Fig. 2.1a, b) and cork stoppers were removed so that males could exit on their own accord. At this stage, dead males were replaced to ensure the same number of bioassay insects (12) for each replicate. To ensure optimal foraging conditions (darkness), a Plexiglas® lid was placed on the top of the arena and covered with a dark plastic sheet, and all external lights were turned off. Six to eight arenas were

set up in the same room that was maintained at a temperature of 25 ± 2 °C for the duration of each bioassay, lasting 15 h.

Treatment stimuli were tested in various combinations against each other, water, or an unbaited (empty) control. Specific amounts of each stimulus were placed in a glass Petri dish (5.0 cm diameter × 1.0 cm high). Liquid test stimuli were quantified in a 4-ml test tube, or when smaller amounts were needed, with a pipette, and were poured directly onto solid stimuli, into a Petri dish, or onto filter paper, depending on the hypothesis being tested.

A baited or an unbaited (control) Petri dish was placed inside an electrical trap constructed from an open aluminum can (15.8 cm diameter × 16.0 cm high) with a 1.5 cm insulated copper ribbon (Fig. 2.1 c; Mistal et al., 2000). When a cockroach contacted the insulated copper ribbon (1st electrode) walking on the inside wall of the can and was still in contact with the metal wall (2nd electrode), it received an electric shock and fell into the bottom of the trap, unable to escape. Traps were placed opposite to one another (180˚) in the arena 5 cm from the wall (Fig. 2.1 b; Mistal et al., 2000). Treatment and control stimuli were randomly assigned to each of the two traps. There were six arenas deployed each bioassay day.

On completion of a bioassay, cockroaches were classified as responders if they were found at the bottom of a trap. After each replicate, the arena and the traps were cleaned with Fisher Sparkleen® Laboratory detergent (2 g; Fisher Scientific Co. Pittsburgh, PA 15219, USA).

2.3.3. Collection of headspace volatiles (HSVs) from bread

Slices of bread (200 g; German rye bread, Mundy Park Bakery, Coquitlam, BC V3J 3R3, Canada) were placed in a Pyrex® glass chamber (34.0 cm high × 12.5 cm wide). A water aspirator drew charcoal-filtered air at 0.5 L min^{-1} for 48 h through the chamber and through a glass tube (6 mm o.d. × 150 mm) containing 200 mg of Porapak-Q™ (50–80 mesh) adsorbent (Byrne et al., 1975). Bread-derived volatiles captured on Porapak-Q were desorbed with 2 ml of pentane. Aliquots of Porapak Q HSV extract

were bioassayed for the response of cockroaches (see below), and the bioactive compounds were identified.

2.3.4. Identification of candidate semiochemicals in attractive HSV extract

Porapak Q HSV extracts shown to be attractive in bioassays were analyzed by gas chromatography (GC) and GC-mass spectrometry (MS), with procedures and equipment previously described (Gries et al., 2002). Briefly, for GC analyses a Hewlett Packard 5890 GC was fitted with a DB-5 GC column (30 m × 0.32 mm i.d.; J & W Scientific, Folsom, CA, USA). Helium was used as the carrier gas (35 cm s^{-1}) with the following temperature program: 50 °C for 5 min, 20 °C min^{-1} to 280 °C. The injector port and flame ionization detector (FID) were set at 250 °C.

Candidate semiochemicals were analyzed by a Saturn 2000 Ion Trap GC-MS operated in full-scan electron impact mode and fitted with a DB-5 GC-MS column (50 m × 0.25 mm i.d.). Using helium as the carrier gas (35 cm s-1), the temperature program was as follows: 50 °C for 1 min, 10 °C min-1 until 280 °C (10 min). The injector port and ion trap were set at 250 and 260 °C, respectively. Compounds were identified by comparing their retention indices in relation to aliphatic hydrocarbons (Van den Dool & Kratz, 1963) and mass spectra with those reported in the literature (Adams, 1989; Jennings & Shibamoto, 1980) and with those of authentic standards (Table 2.2). Those compounds that could not be purchased were synthesized de novo.

2.3.5. Data analyses

All analyses were carried out using SAS statistical software version 9.4. A binary logistic regression model with a Firth bias correction was used to compare mean proportions of responders between test stimuli in each experiment. Type 3 effects for fixed-effect factors (Exps. 1-2, 4-5, 6-8, 9-11, 12-14, 15-17, and 21-28) were used to determine mean differences in the proportion of responders to treatment stimuli between experiments. Post hoc tests with Tukey-Kramer adjustment were used to locate

differences in mean proportions of responders to treatment stimuli between pairs of experiments.

2.3.6. Specific experiments

Attractiveness of bread with water or with beer

Bioassay stimuli consisted of German rye bread (Mundy Park Bakery, Coquitlam, BC V3J 3R3, Canada), beer (Okanagan Springs Pale Ale, Okanagan Spring Brewery, Vernon, BC V1T 9K4, Canada), and water (Burnaby Municipal tap water, BC, Canada). Bioassay stimuli were placed in a glass Petri dish (5.0 cm diam × 1.0 cm high), with an empty Petri dish serving as the control. The treatment or control Petri dish were then randomly assigned to one trap in each pair.

Experiments 1 (*n* = 12) and 2 (*n* = 12) (Table 3) tested the attractiveness of bread (4 g) soaked in water (4 ml) (Exp. 1) or soaked in beer (4 ml) (Exp. 2) *vs* unbaited controls. Experiment 3 (*n* = 14) tested bread (4 g) soaked in water (4 ml) *vs* bread (4 g) soaked in beer (4 ml) to determine whether one stimulus was superior to the other. With either stimulus similarly attractive to GCR males (see Results), experiments 4 (*n* = 12) and 5 (*n* = 12) were then designed to determine whether the attractiveness of bread and water was reliant on bread being soaked in water. Therefore, experiment 4 (*n* = 12) tested bread (4 g) soaked in water (4 ml) *vs* an unbaited control, whereas parallel experiment 5 (*n* = 12) tested bread (4 g) and water (4 ml) in separated Petri dishes within the same trap *vs* an unbaited control.

Potential synergism between bread and water

As both bread in water (Exp. 4), and bread beside water (Exp. 5), strongly attracted GCR males (see Results), Ihad to determine the relative contributions of bread and water to the attractiveness of the 2-component bait. Thus, parallel experiments 6, 7 and 8 (*n* = 12 each) tested water (4 ml) alone, bread (4 g) alone and bread (4 g) soaked in water (4 ml) *vs* unbaited controls. Taking into account that extended periods of water deprivation may alter the insects' propensity to seek water-containing stimuli, two additional sets of three parallel experiments using the same baits as in experiments 6, 7 and 8 bioassayed the responses of GCR males that were water-deprived for 24 h (Exps. 9, 10, 11; *n* = 12 each) or 48 h (Exps. 12, 13, 14; *n* = 12 each) prior to bioassays.

Potential synergism between bread crust and bread crumbs

Bread alone was more attractive than water alone, and as attractive as bread in water (see Results), suggesting that the attractiveness of the bait resided entirely with bread. To determine the relative contribution of bread crust and bread crumbs to the attractiveness of bread, crust (4 g) and crumb (4 g) were tested alone and in binary combination (4 g) *vs* unbaited controls in parallel experiments 15, 16 and 17 (n = 12 each), and against each other in experiment 18 (n = 13).

Attractiveness of bread headspace volatile extract and synthetic bread volatiles

To determine whether the essential semiochemicals that attract GCR males to bread were captured in Porapak headspace volatile extracts of bread (see above), 772 gram-hour equivalents (772 ghe = the amount of volatiles (10 µg) released from 772 g of bread during 1 h) dissolved in pentane (100 µl) were bioassayed *vs* a pentane (100 µl) control (Exp. 19; n = 12). As Porapak Q extract at 772 ghe was attractive to GCR males (see Results), follow-up experiment 20 (n = 24) was designed to determine whether a blend of synthetic bread volatiles at a similar amount could also be an effective trap bait. The blend comprised 16 compounds identified in Porapak headspace volatile extracts of rye bread (Table 2; boldface components) and 28 additional bread odorants reported in the literature (Schieberle & Grosch, 1985, 1987; Chang et al., 1995; Cho & Peterson, 2010) that were not present in Porapak Q extracts. The decision to include these additional bread odorants in the synthetic "Master Blend" (MB) was guided by the objective to compose a bread odorant blend that strongly attracted GCRs rather than to compose a blend that strictly mimicked the odor profile of a specific type of German rye bread. All MB components were prepared at equal proportions except for 3-methylbutanal, acetaldehyde, ethanol, ethyl acetate and acetone which were each admixed at a 10-fold higher dose to account for their extreme volatility. During each bioassay, a 25-µg aliquot of the MB was tested.

With evidence that the 44-component MB (Table 2) was very attractive to GCR males (see Results), follow-up parallel experiments 21-28 (re)tested the attractiveness of the MB (Exp. 21), and the MB lacking certain groups of organic odorants, such as ketones (Exp. 22), aldehydes (Exp. 23), esters (Exp. 24), pyrazines (Exp. 25), alcohols (Exp. 26), furans (Exp. 27), and odorants with multifunctional groups (Exp. 28).

2.4. Results

Attractiveness of bread with water or with beer

Traps baited with bread soaked in water (Exp. 1), or bread soaked in beer (Exp. 2), captured significantly more GCR males than unbaited control traps (Exp. 1: $z = 6.57$, $P<0.001$; Exp. 2: $z = 7.23$, $P<0.001$; Fig. 2.2). Paired traps baited with bread soaked in water or with bread soaked in beer were equally effective in capturing GCR males (Exp. 3: $z = 0.08$, $P = 0.94$; Fig. 2.2). Traps baited with bread soaked in water (Exp. 4), or bread beside water (Exp. 5), each captured significantly more GCR males than unbaited control traps (Exp. 4: $z = 6.31$, $P<0.001$; Exp. 5: $z = 6.02$, $P<0.001$; Fig. 2.2). The proportions of GCR males responding to treatment stimuli in experiments 4 and 5 (bread in water and bread beside water, respectively) did not differ significantly (Tukey-Kramer; $z = 0.75$, $P = 1.005$).

Potential synergism between bread and water

When GCR males were not water-deprived, greater proportions of males were captured in traps baited with water (Exp. 6), bread (Exp. 7) or bread in water (Exp. 8) than in unbaited control traps (Exp. 6: $z = 4.09$, $P<0.001$; Exp. 7: $z = 6.72$, $P<0.001$; Exp. 8: $z = 6.09$, $P<0.001$; Fig. 2.3). The proportions of GCR males responding to treatment stimuli in experiments 6-8 did not differ.

When GCR males were water-deprived for 24 h, water-baited traps and unbaited control traps captured similar proportions of males (Exp. 9: $z = 1.50$, $P = 0.13$; Fig. 3). In contrast, traps baited with bread (Exp. 10), or with bread in water (Exp. 11), each captured significantly greater proportions of males than unbaited control traps (Exp. 10: $z = 6.69$, $P<0.001$; Exp. 11: $z = 4.80$, $P<0.001$; Fig. 2.3). The proportions of GCR males captured in traps baited with water (back-transformed mean logit ± SE: 0.58 ± 0.05), bread (0.84 ± 0.03) or bread in water (0.72 ± 0.04) differed significantly. Bread-baited traps captured significantly greater proportions of males than water-baited traps (Tukey-Kramer, $P = 0.0083$).

When GCR males were water-deprived for 48 h, water-baited traps and unbaited control traps captured similar proportions of males (Exp. 12: $z = 1.77$, $P = 0.08$; Fig. 2.3). In contrast, traps baited with bread (Exp. 13), or bread in water (Exp. 14), each captured significantly greater proportions of males than unbaited control traps (Exp. 13: $z = 6.14$,

P<0.001; Exp. 14: z = 6.29, P<0.001; Fig. 2.3). The proportions of GCR males captured in traps baited with water (back-transformed mean logit ± SE: 0.59 ± 0.05), with bread (0.84 ± 0.04), and with bread in water (0.83 ± 0.048) did not differ significantly.

Potential synergism between bread crust and bread crumbs

Traps baited with bread crust (Exp. 15), bread crumbs (Exp. 16) or bread crust and crumbs (Exp. 17), each captured significantly greater proportions of GCR males than unbaited control traps (Exp. 15: z = 6.12, P<0.001; Exp. 16: z = 6.72, P<0.001; Exp. 17: z = 6.51, P<0.001). The proportions of GCR males captured in traps baited with bread crust (back-transformed mean logit ± SE: 0.83 ± 0.04), bread crumbs (0.90 ± 0.03) and bread crust and crumbs (0.85 ± 0.03) did not differ. Traps baited with bread crust captured significantly greater proportions of GCR males than traps baited with bread crumbs (Exp. 18: z = 2.92, P = 0.0035).

Identification of candidate semiochemicals in attractive HSV extract

Sixteen compounds were identified in HSV extract, including aldehydes, esters, alcohols, ketones furans, and pyrazines (Table 2).

Attractiveness of bread headspace volatile extract and synthetic bread volatile blends

Traps Traps baited with Porapak Q headspace volatile extract of bread captured significantly greater proportions of GCR males than traps with a pentane control stimulus (Exp. 19: z = 6.63, P<0.001). Similarly, traps baited with the synthetic MB (Table 2) captured significantly greater proportions of GCR males than traps treated with a pentane control stimulus (Exp. 20: z = 8.53, P<0.001). In parallel experiments 21-28, traps baited with the MB captured significantly greater proportions of GCR males than unbaited control traps (Exp. 21: z = 7.70, P<0.001), as did traps baited with the MB lacking ketones (Exp. 22: z = 8.22, P<0.001), aldehydes (Exp. 23: z = 7.77, P<0.001), esters (Exp. 24: z = 7.56, P<0.001), pyrazines (Exp. 25: z = 5.12, P<0.0001), alcohols (Exp. 26: z = 6.14, P<0.001), furans (Exp. 27: z = 6.43, P<0.001) or volatiles with multifunctional groups (Exp. 28: z = 4.56, P<0.001). The proportions of GCR males captured in treatment traps of experiments 21-28 did not significantly differ), indicating that all synthetic blends were equally effective trap baits, and that no one single functional group of volatiles had a significant effect on the blend's attractiveness.

2.5. Discussion

Our data show that (1) bread is a highly attractive food source for GCRs; (2) neither water nor

beer enhance the attractiveness of bread; (3) bread crust is more attractive than bread crumbs; and (4) a blend of synthetic bread odorants is as effective as natural bread in attracting GCRs.

As omnivores, GCRs feed on many types of food, including carbohydrates, animal and plant proteins, lipids, vegetables and fruit (Table 1). Their food preference varies in accordance with their stage of development (Kunkel, 1966), sex and reproductive status (Durbin & Cochrane, 1985; Silverman, 1986), population density (Silverman, 1986), activity level (Metzger, 1995), the travel distance to food patches (Silverman, 1986), water-deprivation (Cornwell, 1968; Ross, 1981), the composition of their prior meal (Kells & Bennet, 1998; Jones & Raubenheimer, 2001; Raubenheimer & Jones, 2006), and potential nutritional deficiencies (Kells & Bennett, 1998). It follows that the ideal GCR bait should consist of a complete or balanced diet (Wolfe et al., 1997), that contains any food type that would satisfy all of the GCRs' varied nutritional needs.

Bread and beer contain highly diverse and nutritious ingredients (Bamforth, 2002; Dewettinck et al., 2008) and are very attractive to foraging GCRs (Table 1; Rau 1945; Ebeling et al. 1966; Ebeling & Reierson, 1974; Reierson & Rust, 1977; Reierson et al., 1979; Ballard & Gold, 1982; Wileyto & Boush, 1983). We predicted that the bread-in-beer combination would be superior to the attractiveness of bread, or of beer, because GCRs need food (specifically carbohydrates) and water for survival (Cornwell, 1968; Ross 1981; Carrel & Tanner, 2002) and because the water-induced microbial breakdown of bread nutrients should amplify the overall bouquet of the bread-in-beer food source. Unexpectedly, bread-in-beer was not more attractive to GCRs than bread-in-water (Fig. 2.2; Exps. 1, 2, 3), or bread alone (Fig. 2.3), even when GCRs were water-deprived for up to 48 h (Exps. 4-14). It is conceivable that the 24-h to 48-h time period of water-deprivation was insufficient in the relatively humid rearing rooms to reveal the importance of water for GCRs as an essential foraging resource.

To determine the relative attractiveness of bread crust and bread crumbs to GCRs, we again worked with rye bread as a "model" source. Unlike roasted white bread

which has a simple popcorn-like aroma (Schieberle & Grosch, 1987), rye bread has a diverse odor profile with malty-, green-, tallow-, sweet-, cereal- and potato-like odors. Moreover, the odor profiles of rye bread crust and crumbs differ as a result of Maillard reactions occurring during baking (Schieberle & Grosch, 1994). The crust emanates larger amounts of 3-methylbutanal, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, maltol and three pyrazines than crumbs, whereas crumbs emanate larger amounts of phenylacetaldehyde and hexanal than crust (Schieberle & Grosh, 1994). As predicted, bread crust attracted significantly more GCRs than did bread crumbs (Fig. 2.4; Exp. 18). Despite the distinctly different odor profiles of bread crust and crumbs, there was no synergistic effect on attraction of GCRs (Fig. 2.4; Exps. 15-17).

As natural food baits can perish over time, produce off-putting odors and lose effectiveness, we also wanted to identify the key semiochemicals of bread that attract GCRs for the potential development of a synthetic lure. With evidence that Porapak Q headspace volatile extract of rye bread is attractive to GCRs (Fig. 2.5; Exp. 1), we identified all odorants in that extract. As the ultimate synthetic lure ought to be optimally attractive to GCRs, we compiled a synthetic blend that contained not only the specific odorants of the rye bread we bioassayed in our study (Figs. 2.2-2.4), but added also bread odorants reported in the literature (Schieberle & Grosch, 1985, 1987, 1994; Chang et al., 1995; Cho & Peterson, 2010). As expected, this Master Blend (Table 2) proved highly attractive to GCRs (Fig. 2.5; Exp. 20). Unexpectedly, however, there was astounding redundancy in the semiochemical blend. The blend's effectiveness was not linked to the presence of any one of seven groups of organic molecules such as furans or pyrazines (Fig. 2.5; Exps. 21-28). Even a partial Master Blend lacking three groups of organic molecules (aldehydes, ketones, multifunctional compounds) was still effective (data not shown). These results indicate that a specific set of bread semiochemicals can compensate for the absence of others. The minimum number of organic groups to be represented in a blend, and the minimum number of blend components needed to attract GCRs, are yet to be determined.

Our choice to bioassay GCR males instead of females in all experiments was guided by reports that males are the most active foragers, followed by virgin, mated and ootheca-bearing females (Metzger, 1995; but see DeMark & Bennett, 1995). We did not consider bioassaying first instar nymphs because they hardly forage (Kopanic & Schal, 1999) and rely on coprophagy for survival (Kopanic et al., 2001). Even though the

foraging activity of nymphs intensifies with increasing instars (Metzger, 1995), it still seemed to lag behind that of males (personal observation). We are confident that females and mid- to late-instar nymphs respond similarly, based on a parallel food preference study with GCRs, where males, females and nymphs exhibited comparable behavioral responses (JP, unpublished).

We conclude that rye bread is a highly effective attractant for GCRs. It could be used as a bait in retainer traps, or, laced with insecticide, as a food source in bait stations. Although the presence of water did not alter capture rates of GCRs in our experiments, water might enhance the attractiveness of bread in arid climates. A lure of synthetic bread semiochemicals may eventually replace bread as a bait in retainer traps, but the minimum number of essential semiochemicals for that lure has yet to be determined.to be determined.

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Table 2.1 *Natural food types reported or recommended for attraction of German cockroaches*

Carbohydrates	
Specific sources	**References**
Beer	Mallis, 1969; Wileyto & Boush, 1983
Raisins	Robinson et al., 1980; Akers & Robinson, 1981; Rust & Reierson, 1981; Ballard & Gold, 1982; Stauffer, 2007
Bread	Rau, 1945; Ebeling et al., 1966; Ebeling & Reierson, 1974; Reierson & Rust, 1977; Reierson et al., 1979; Ballard & Gold, 1982; Wileyto & Boush, 1983; Stauffer, 2007
Cinnamon bun	Rau, 1945
Fresh potato and sugar paste	Miesch, 1964
Rice bran	Tsuji, 1965; Doi & Nakagaki, 1987
Potato	Piper et al., 1975; Ballard & Gold, 1982; Brenner & Patterson, 1988; Stauffer, 2007
Extracts of Coca-Cola and other soft drink syrups	Lofgren & Burden, 1958; Reierson & Rust, 1977; Schal & Hamilton, 1990
Corn and maple syrup	Barak et al., 1977; Spaulding & Pararela, 1989; Bruey, 1991a; Brenner et al., 1991; Geary, 1992; Wolfe et al., 1997
Honey or molasses	Bare, 1945; Spaulding & Pasarela, 1989; Bruey, 1991b
Sugar	Bare, 1945; Lofgren & Burden, 1958; Broadbent, 1977; Wileyto & Boush, 1983; Stapleton & Stapleton, 1994; Stauffer, 2007
Fructose	Tsuji, 1965; Silverman & Bieman, 1996
Maltose	Tsuji 1965; Lofgren and Burden 1958; Broadbent 1977
Distiller's grain	Brenner & Patterson, 1988; Brenner et al., 1991; Brenner & Burns, 1999; Nalyanya & Schal, 2001
Oatmeal	Spaulding & Pasarela, 1989; Geary, 1992
Proteins	
Rat chow	Tsuji, 1965; Valles et al., 1996
Dog food	Olton, 1975; Ballard & Gold, 1982
Dog food	Olton, 1975; Ballard & Gold, 1982
German cockroaches and German cockroach feces	Ishii & Kuwahara, 1968; Ballard & Gold, 1982
Dry yeast	Ballard & Gold, 1982
Animal proteins	Rau, 1945
Peanut butter	Nalyanya & Schal, 2001; Karimifar, 2009
Protein sources derived from poultry liver, silkworm pupae and hydrogenated soy protein	Wolfe et al., 1997
Lipids	
Butter	Bare, 1945

Table 2.1 *continued …*

Saturated or unsaturated fatty acids, alone or in combinations	Ong, 1989
Corn oil	Lofgren & Burden, 1958; Wolfe et al., 1997
Fruits/Vegetables/Fibre	
Fresh apple	Piper & Frankie, 1978; Ballard & Gold, 1982; Stauffer, 2007
Banana	Reierson & Rust, 1977; Piper et al., 1975; Ballard & Gold, 1982
Osage orange	Ballard & Gold, 1982
Yellow onions	Stapleton & Stapleton, 1994
Pre-gelatinized tapioca and wheat starch	Brenner & Burns, 1999
Essential Oils	
Banana, sweet orange, apple and pineapple	Schal & Hamilton, 1990 (and references cited therein)
Funugreek seed	Wileyto & Boush, 1983

Table 2.2 Bread odorants arranged by functional group comprising the Master Blend (MB) bioassayed for attraction of German cockroaches. Boldface compounds were identified in headspace volatile extracts of rye bread. Other bread odorants are reported in the literature; RI = retention indices (Van den Dool & Kratz, 1963) relative to aliphatic hydrocarbons.

Groups of organic chemicals	*RI*	Amount (per µl) in MB	Supplier
Aldehydes			
hexanal	*801*	5.6	Sigma-Aldrich Co.[a]
benzaldehyde	*970*	5.6	Sigma-Aldrich Co.[a]
phenylacetalaldehyde	*1050*	5.6	Sigma-Aldrich Co.[a]
nonanal	*1106*	5.6	Sigma Chemical Co.[c]
3-methylbutanal	*655*	56.0	Sigma-Aldrich Co.[a]
(*Z*)-4-heptenal	*899*	5.6	Sigma-Aldrich Co.[a]
(*E*)-2-heptenal	*963*	5.6	Aldrich Chemical Co.[b]
(*E*)-2-octenal	*1063*	5.6	Bedoukian Research Inc.[d]
(*E*)-2-nonenal	*1164*	5.6	Bedoukian Research Inc.[d]
(*E*,*Z*)-2,6-nonadienal	*1157*	5.6	Aldrich Chemical Co.[b]
acetylaldehyde	*419*	56.0	Sigma Chemical Co.[c]
2-methylpropanal	*556*	5.6	Aldrich Chemical Co.[b]
(*E*,*E*)-2,4-decadienal	*1323*	5.6	Aldrich Chemical Co.[b]
Esters			
butyl butanoate	*997*	5.6	SFU[e]
ethyl hexanoate	*999*	5.6	SFU[e]
hexyl butanoate	*1192*	5.6	SFU[e]
ethyl octanoate	*1196*	5.6	SFU[e]
ethyl acetate	*615*	56.0	SFU[e]
methylhexanoate	*929*	5.6	SFU[e]
Alcohols			
hexan-1-ol	*875*	5.6	Sigma-Aldrich Co.[a]
1-octen-3-ol	*984*	5.6	Sigma-Aldrich Co.[a]
ethanol	*465*	56.0	Commercial Alcohols[f]
3-methylbutanol	*736*	5.6	Ana chemia[g]
phenylethyl alcohol	*1117*	5.6	Fluka[h]

Table 2.2 continued…

Ketones			
1-octen-3-one	982	5.6	SFU[e]
2-heptanone	892	5.6	Sigma Chemical Co.[c]
acetone	493	56.0	Sigma-Aldrich Co.[a]
2-octanone	992	5.6	SFU[e]
Furans			
furan-2-carbaldehyde	837	5.6	Sigma-Aldrich Co.[a]
2-furanmethanol	859	5.6	Sigma-Aldrich Co.[a]
2-acetylfuran	909	5.6	Sigma-Aldrich Co.[a]
5-(hydroxymethyl)-2-furaldehyde	1226	5.6	Sigma-Aldrich Co.[a]
5-methylfuran	966	5.6	Sigma-Aldrich Co.[a]
Pyrazines			
2,6-dimethylpyrazine	913	5.6	Aldrich Chemical Co.[b]
2,6-dimethyl-3-ethylpyrazine	1085	5.6	SFU[e]
2-methyl-3-ethylpyrazine	1000	5.6	Sigma-Aldrich Co.[a]
ethylpyrazine	917	5.6	Sigma-Aldrich Co.[a]
Multi-functional			
ethyllactate	813	5.6	Aldrich Chemical Co.[b]
2,3-butandione	593	5.6	SFU[e]
pyruvic aldehyde	593	5.6	SAFC Supply Solutions[i]
3-hydroxy-2-butanone	710	5.6	TCI America[j]
methional	909	5.6	Sigma-Aldrich Co.[a]
2-methoxy-4-vinylphenol	1315	5.6	Penta Manufacturing[k]
2-methoxyphenol	1088	5.6	Fluka[h]

[a] – Sigma-Aldrich Co., St. Louis, MO, USA. [b] – Aldrich Chem Co., Milwaukee, WI, USA. [c] – Sigma Chemical Co., St. Louis, MO, USA. [d] – Bedoukian Research Inc., Danbury, CT, USA. [e] – Gries-laboratory, Simon Fraser University, Burnaby, BC, CAN. [f] – Commercial Alcohols, Brampton, ON, CAN. [g] – Ana chemia – Anachemia Canada Inc., Montreal, QU, CAN. [h] – Fluka, Fluka Chemie, Buchs, CH. [i] – SAFC Supply Solutions, St. Louis, MO, USA. [j] – TCI America, Tokyo Chemical Industry Co. Ltd., Kita Ku, Tokyo, Japan. [k] – Penta Manufacturing – Penta International Corp., Livingston, NJ, USA. [l] – Caledon Laboratory Ltd., Georgetown, ON, CAN

Table 2.3 *Stimuli tested for the responses of German cockroach males in still-air arena olfactometer (Figure 2.1) bioassays.*

Exp.[1]	*n*[2]	H_2O deprivation (h)	Stimulus 1	Stimulus 2
Objective 1: Test for attractiveness of bread with water or bread with beer				
1	12	0	Bread[3] (4 g) in water (4 ml)	Unbaited
2	12	0	Bread (4 g) in beer (4 ml)[4]	Unbaited
3	14	0	Bread (4 g) in beer (4 ml)	Bread (4 g) in water (4 ml)
Objective 2: Potential synergism between bread and water				
4	12	0	Bread (4 g) in water (4 ml)	Unbaited
5	12	0	Bread (4 g) *plus* water (4 ml)	Unbaited
6	12	0	Water (4 ml)	Unbaited
7	12	0	Bread (4 g)	Unbaited
8	12	0	Bread (4 g) in water (4 ml)	Unbaited
9	12	24	Water (4 ml)	Unbaited
10	12	24	Bread (4 g)	Unbaited
11	12	24	Bread (4 g) in water (4 ml)	Unbaited
12	12	48	Water (4 ml)	Unbaited
13	12	48	Bread (4 g)	Unbaited
14	12	48	Bread (4 g) in water (4 ml)	Unbaited
Objective 3: Potential synergism between bread crust and bread crumb				
15	12	48	Crust (4 g)	Unbaited
16	12	48	Crumbs (4 g)	Unbaited
17	12	48	Bread (4 g)	Unbaited
18	13	48	Crust (4 g)	Crumbs (4 g)
Objective 4: Attractiveness of bread headspace volatile extract and synthetic bread volatiles				
19	12	48	HSV of bread[5] (100 µl)	Pentane (100 µl)
20	24	48	MB[6] (50 µl)	Pentane (50 µl)
21	22	48	MB (50 µl)	Pentane (50 µl)
22	20	48	MB *minus* ketones (50 µl)	Pentane (50 µl)
23	18	48	MB *minus* aldehydes (50 µl)	Pentane (50 µl)
24	21	48	MB *minus* esters (50 µl)	Pentane (50 µl)
25	18	48	MB *minus* pyrazines (50 µl)	Pentane (50 µl)
26	20	48	MB *minus* alcohols (50 µl)	Pentane (50 µl)
27	20	48	MB *minus* furans (50 µl)	Pentane (50 µl)
28	8	48	MB *minus* multi-functionals (50 µl)	Pentane (50 µl)

[1] Exps. 1-2, 4-5, 6-8, 9-11, 12-14, 15-17, and 21-28 were run in parallel; [2] each replicate (*n*) tested 12 48-h starved males; [3] German rye bread, Mundy Park Bakery, Coquitlam, BC, Canada; [4] Okanagan Spring Pale Ale, Okanagan Spring Brewery, BC, Canada; [5] headspace volatile (HSV) extract in 100 µl of pentane was tested at 772 gram-hour equivalents (772 ghe = the amount of volatiles (10 µg) given off 772 g of bread during 1 h); [6] Master Blend (MB) composition listed in Table 2; a 25-µg aliquot in 50 µl of pentane was tested in each bioassay.

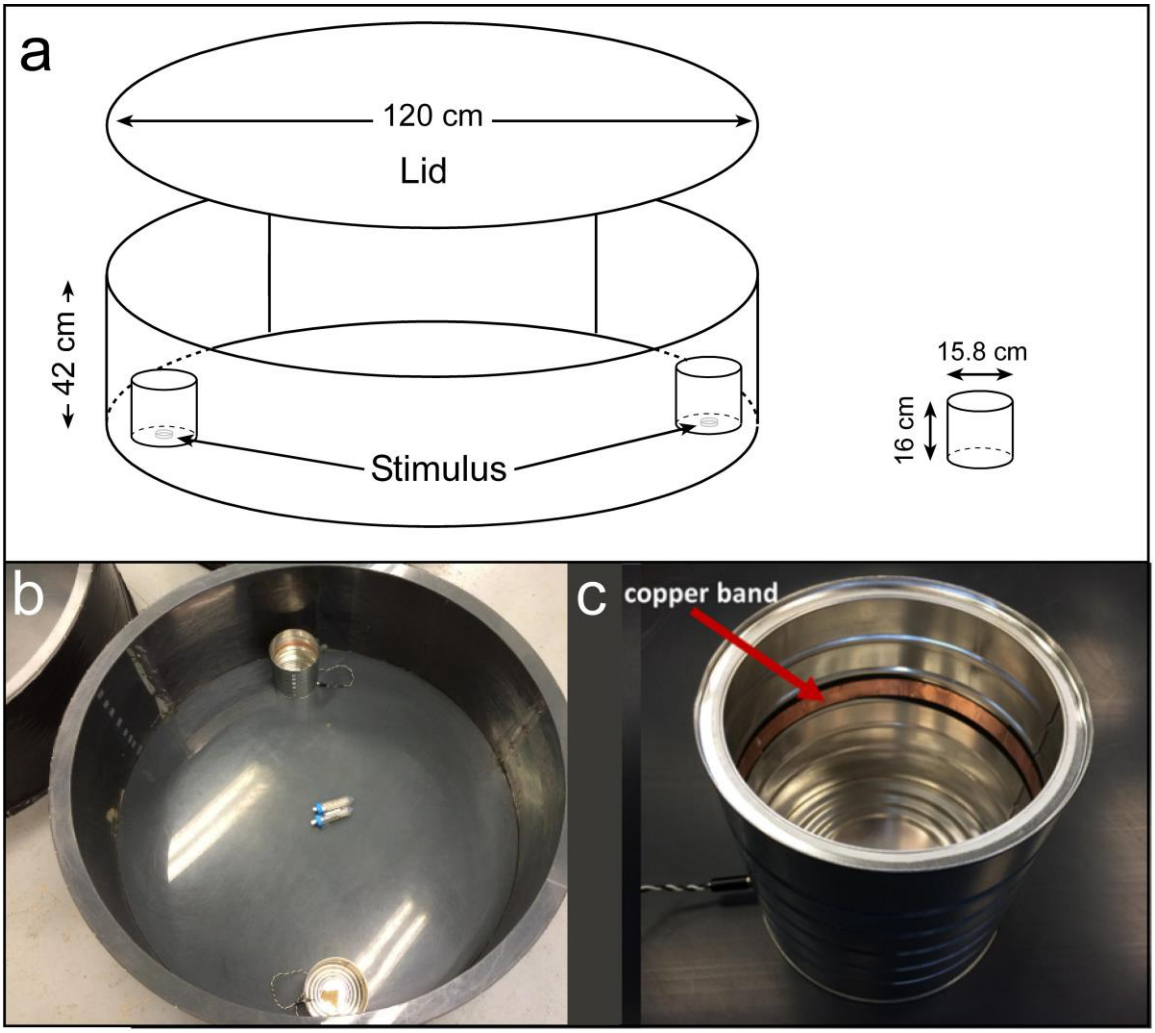

Figure 2.1 *Schematic and photographic illustrations of the two-choice bioassay arena, with metal cans modified to serve as cockroach traps. When a cockroach contacted the insulated copper ribbon (1st electrode) walking on the inside wall of the can and was still in contact with the metal wall (2nd electrode), it received an electric shock and fell into the bottom of the trap, unable to escape (adapted from Mistal et al., 2000).*

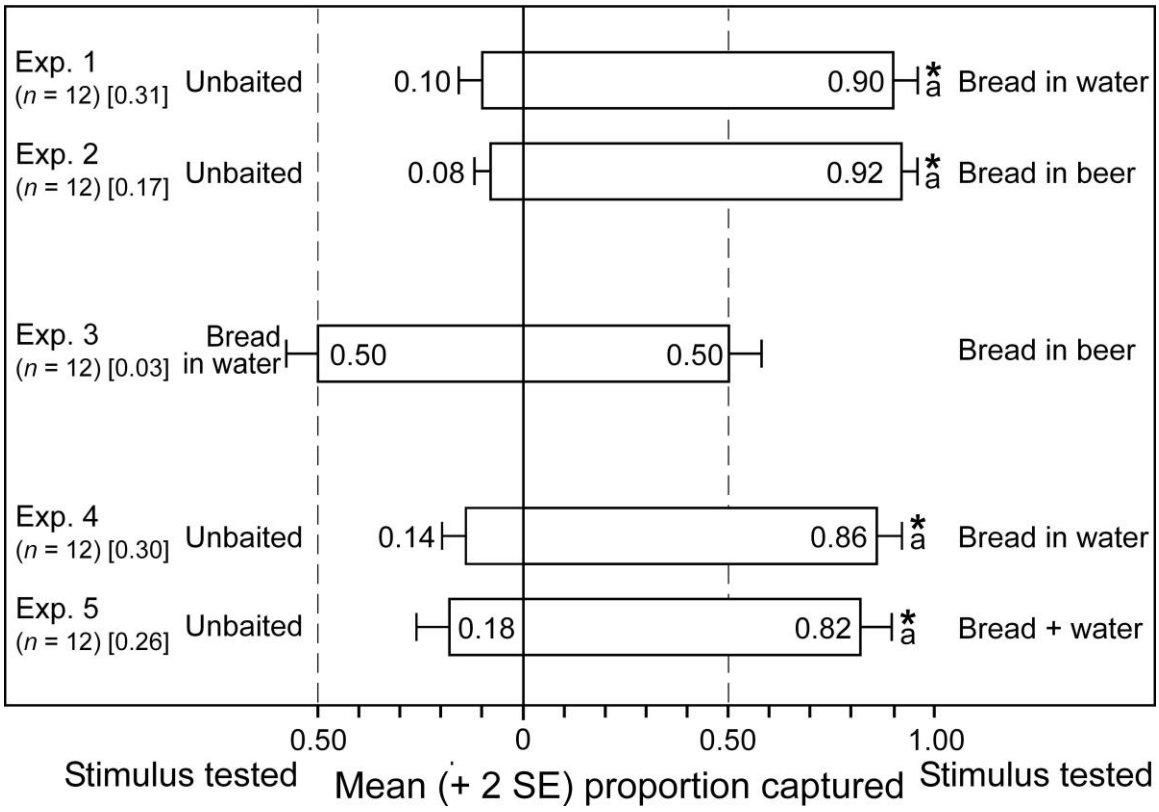

Figure 2.2 *Back-transformed mean (+ 2SE) proportions of Blattella germanica males captured in two-choice arena bioassays (Figure 1) in traps baited with various test stimuli or left unbaited (Table 3). An asterisk on a bar denotes a significantly higher proportion of trap captures induced by the respective trap bait (P<0.0001). Grouped experiments were run in parallel; proportions of captures in treatment traps did not differ among parallel experiments 1-2 and 4-5; n indicates the number of replicates; numbers in square brackets indicate the mean proportions of males not captured in treatment or control traps.*

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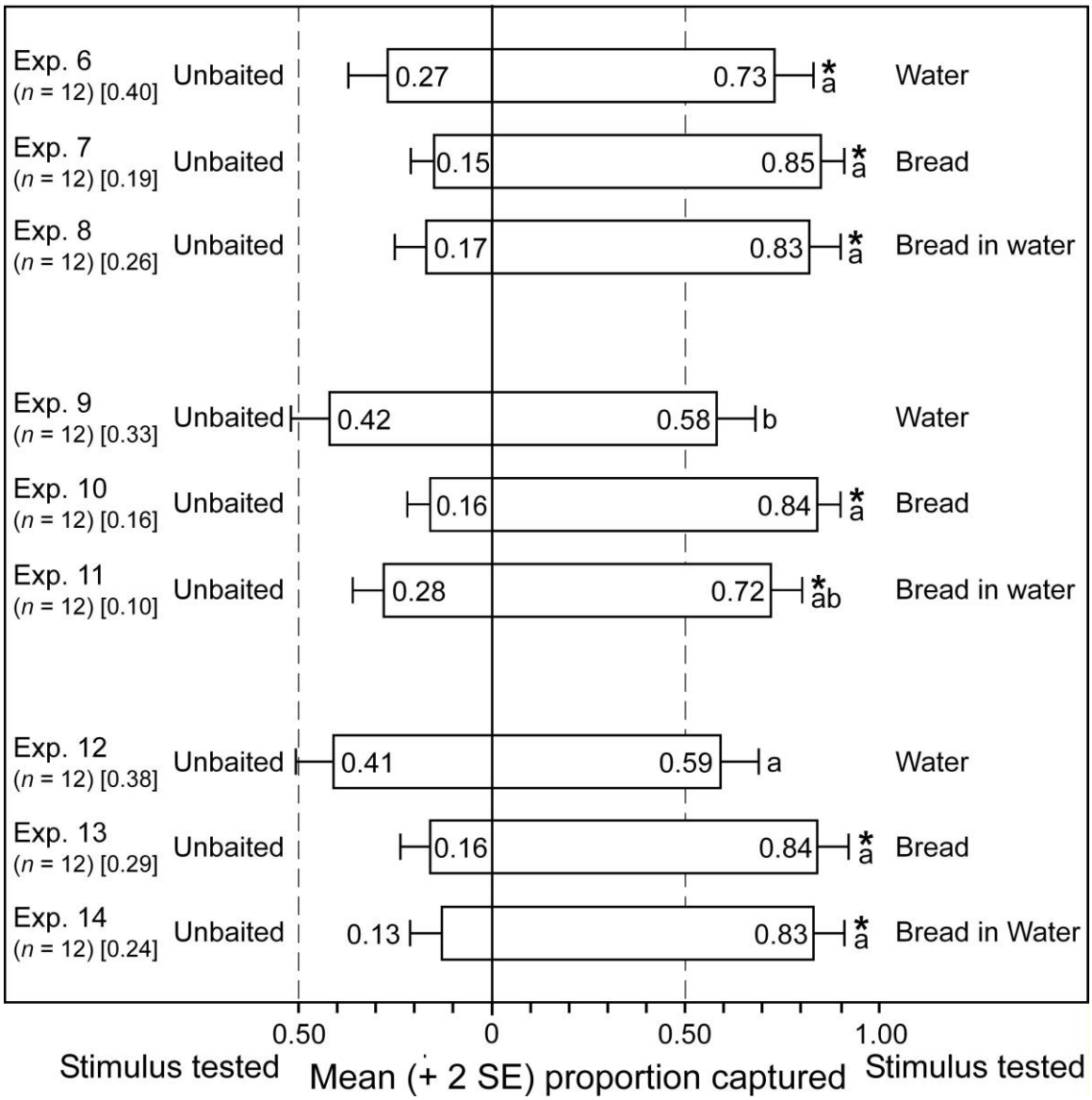

Figure 2.3 *Back-transformed mean (+ 2SE) proportions of Blattella germanica males captured in two-choice arena bioassays (Figure 1) in traps baited with water, bread, or bread in water, or left unbaited (Table 3). Males in parallel experiments 6-8, 9-11 and 12-14 were water-deprived for 0 h, 24 h and 48 h, respectively, prior to bioassays. An asterisk on a bar denotes a significantly greater proportion of trap captures induced by the respective trap bait (P<0.0001). Proportions of captures in treatment traps did not differ among experiments 6-8 or 12-14, but differed among experiments 9-11 as indicated by different letter superscripts on bars (Tukey-Kramer, P<0.05); n indicates the number of replicates; numbers in square brackets indicate the mean proportions of males not captured in treatment or control traps.*

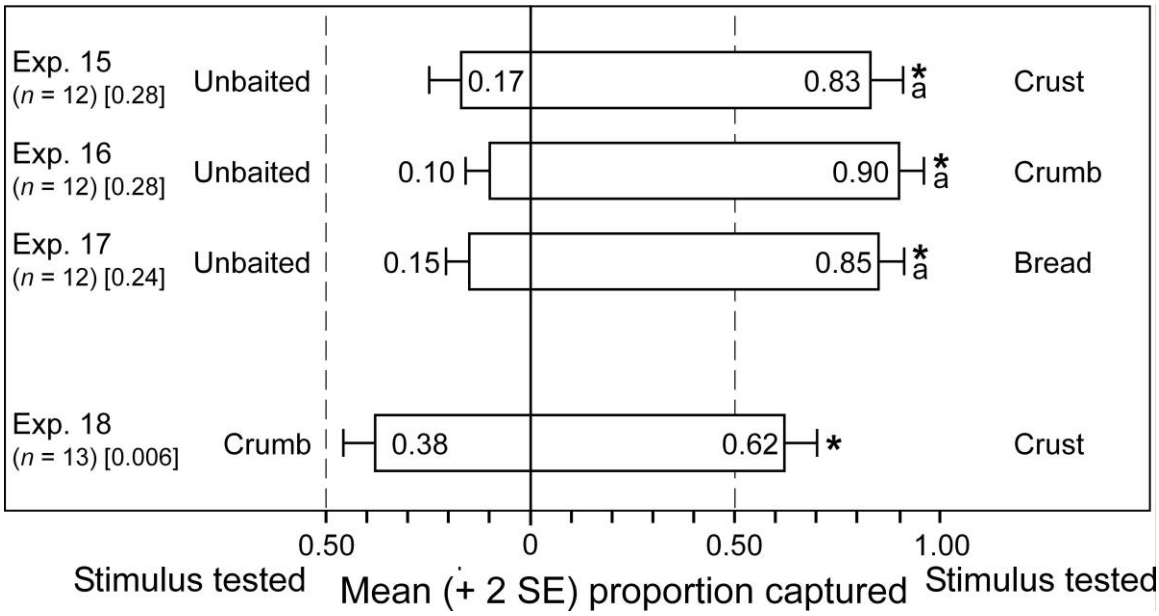

Figure 2.4 *Back-transformed mean (+ 2SE) proportions of Blattella germanica males captured in two-choice arena bioassays (Figure 1) in traps baited with bread crust, bread crumbs or both, or left unbaited (Exps. 15-17), or captured in traps baited with either bread crust or bread crumbs (Exp. 18). An asterisk on a bar denotes a significantly greater proportion of trap captures induced by the respective trap bait [P<0.0001 (Exps. 15-17), P<0.05 (Exp. 18)]. Proportions of captures in treatment traps did not differ among parallel experiments 15-17; n indicates the number of replicates; numbers in square brackets indicate the mean proportions of males not captured in treatment or control traps.*

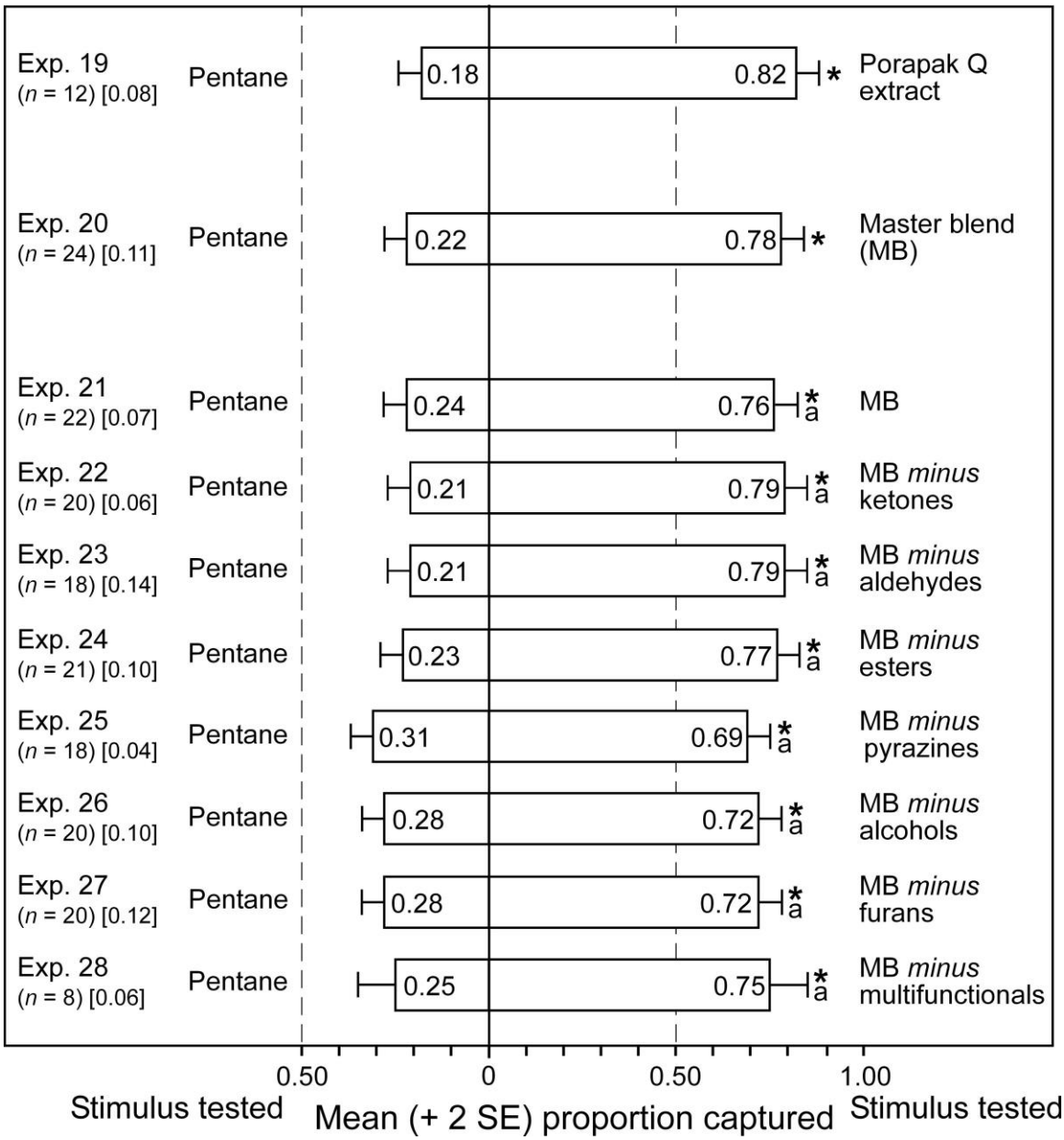

Figure 2.5 *Back-transformed mean (+ 2SE) proportions of Blattella germanica males captured in two-choice arena bioassays (Figure 1) in traps baited with (i) a Porapak Q extract of bread headspace volatiles [772 gram-hour-equivalents (10 µg); Exp. 19], (ii) a Master blend (MB, 25 µg in 50 µl of pentane) of synthetic bread odorants (Table 2) (Exp. 20), and (iii) the complete MB (Exp. 21) and MBs lacking certain groups of organic chemicals (Exps. 22-28). An asterisk on a bar denotes a significantly greater proportion of trap captures induced by the respective trap bait (P < 0.0001). Proportions of captures in treatment traps did not differ among parallel experiments 21-28; n indicates the number of replicates; numbers in square brackets indicate the mean proportions of males not captured in treatment or control traps.*

Chapter 3. New Food Baits for Trapping German cockroaches, *Blattella germanica* (L.)[1]

[1]The corresponding manuscript has been accepted for publication in the Journal of Economic Entomology, with the following authors: Joshua C. Pol, Sebastian Ibarra Jimenez, and Gerhard Gries

3.1. Abstract

German cockroaches (GCRs), Blattella germanica (Dictyoptera: Blattellidae), are attracted to those beer semiochemicals (e.g., ethanol) that formerly living and active yeasts have produced or that otherwise formed in the brewing process. We predicted that an earlier step in the production of beer, where yeasts actively metabolize the sugar in malted barley powder (dry malt extract = DME), is very attractive to GCRs. In laboratory experiments, a 3-component composition (3CC) comprising DME, water, and Brewer's yeast strongly attracted GCR nymphs, females, and males. Both Brewers' yeast and "spoilage organisms" in the DME or water seem to add to the attractiveness of the 3CC but there is no additive or synergistic effect between them. The 3CC becomes optimally attractive to GCRs after 12 h of fermentation and stays that attractive for at least 120 h. In field trapping experiments, the 3CC and - unexpectedly - also the DME each proved as effective for attracting and capturing GCRs as a commercial cockroach bait (Combat Roach Gel). Future studies will investigate lethal biocontrol agents that can be added to the 3CC, or the DME, and will explore the efficacy of such lethal baits for GCR control.

Key words: German cockroaches, dry malt extract, Brewer's yeast, attraction, commercial cockroach bait

3.2. Introduction

German cockroaches (GCRs), *Blattella germanica* L. (Dictyoptera: Blattellidae), are widespread and prolific urban insect pests. As true omnivores, they feed on a wide range of food resources including feces, manure, organic waste and many products prepared for animal and human consumption (Schal and Hamilton 1990). Thus, GCRs may acquire, carry, and spread a plethora of microbes that cause human illnesses (Solomon et al. 2016). Moreover, GCR-derived allergens in household dust cause asthma and allergic diseases in inner-city children (Do et al. 2016).

The efficacy of GCR abatement programs is strongly dependent upon attractants that lure GCRs into retainer traps or to insecticide-laced baits (Schal and Hamilton 1990). Searches for these attractants have focused on food types and their odorants that GCRs frequently seek or preferentially feed on, such as beer and bread (e.g., Reierson et al. 1979). Noteworthy, both beer-brewing and bread-baking involve yeasts that metabolize nutrients and, in the process, produce specific fermentation odorants such as ethanol that attract GCRs (Karifimar et al. 2011).

Brewer's yeast, *Saccharomyces cerevisiae,* is the predominant yeast species used for commercial production of food and beverages, such as bread, sake, wine, and ale (Sicard and Legras 2011). The polyphyletic group of domesticated industrial strains of *S. cerevisiae* differs from wild strains mainly in its ability to ferment sugars, undergo sexual reproduction, and produce aromatic odorants (Gallone et al. 2016).

Brewer's yeast has been shown to emit semiochemicals (message bearing chemicals) that attract insect vectors (Christiaens et al. 2014). For example, *S. cerevisiae* produces a blend of isopentyl acetate and ethyl acetate that attracts the vinegar fly *Drosophila melanogaster* (Christiaens et al. 2014), and a blend of isopentyl acetate, ethyl hexanoate, and 3-methyl butan-1-ol that attracts the yellowjacket *Vespula vulgaris* (Brown et al. 2014).

Here we tested the hypothesis that yeast contribute to the attractiveness of a food source (beer) that GCRs preferentially seek and consume (Cochran 1999). We focused on beer because it is a well-known "home remedy" bait for GCR trapping, and the yeast-produced ethanol of beer has already been reported as a semiochemical attractant for GCRs (Karimifar et al. 2011). Interestingly, most types of bottled or canned beer do not

contain any live yeasts and the attraction of GCRs to (stale) beer is based on those semiochemicals (e.g., ethanol) that formerly living and active yeasts have produced, or that otherwise formed in the brewing process (Karimifar et al. 2011). It follows that an ingredient (Brenner and Patterson 1988; Brenner and Patterson 1989), or an earlier step in the production of beer (for a review see Lodolo et al. 2008), where yeasts actively metabolize the sugars in malted barley powder (dry malt extract (DME)), might be at least as attractive to GCRs as the actual beer beverage.

A fermenting 3-component composition (3CC) comprising DME, water and Brewer's yeast as a potential bait for GCRs would have the advantage of providing not only food but also water to foraging GCRs. Access to moist food would be particularly important to GCRs in arid habitats. It is also conceivable that water, or the DME, on its own is attractive to GCRs. During both the kiln drying process and the boiling of wort, Maillard reactions between amino acids and reducing sugars take place (Lodolo et al. 2008) that may produce one or more semiochemicals such as 2,3-dihydro-3,5-dihydro-6-methyl-4*H*-pyran-4-one which – together with ethanol – is known to attract GCRs (Karimifar et al. 2011).

Limited longevity of the 3CC is a potential disadvantage that may preclude deployment of the composition as a GCR bait. Once water is added to the composition and yeasts are activated, they may quickly consume all the nutrients, then die and render the composition ineffective for GCR attraction. Therefore, it was important to determine the "field-life" of the composition.

Our objectives were to (1) determine the attractiveness of the 3CC to juvenile and adult GCRs; (2) study the effect(s) of Brewer's yeasts and yeast contaminants on 3CC attractiveness; (3) investigate the effect of fermentation period (6-120 h) on 3CC attractiveness; (4) compare the 3CC attractiveness to that of commercial GCR baits; and (5) compare the efficacy of the 3CC, DME, and a commercial bait for field trapping GCRs.

3.3. Materials and Methods

3.3.1. Experimental Insects

A GCR colony was established in 2004 (Karimifar et al. 2011) and supplemented with specimens captured in apartment buildings in Vancouver (British Columbia (BC), Canada) and adjacent municipalities. The colony was reared in the Insectary Annex of Simon Fraser University, Burnaby, BC. GCRs were kept in Plexiglas® cages (30 cm wide × 60 cm long × 45 cm high) fitted with two mesh-covered openings for air circulation, and maintained at 25 ± 1 °C and a 40-70% relative humidity under a photoperiod of L14:D10. Panels of narrowly spaced particle board and paper towel coverings provided shelters. The diet consisted of Purina® Dog Food (Purina Canada, Mississauga, Can), fresh apple slices and water.

3.3.2. General Bioassay Procedure

Bioassays were conducted in cylindrical Plexiglas® still-air arenas (120 cm diameter × 42 cm high) (Mistal et al. 2000). A baited or an unbaited (control) Petri dish (5.0 cm diameter × 1.0 cm high) was placed inside an electrical trap constructed from an open aluminum can (15.8 cm diameter × 16.0 cm high) with a 1.5 cm insulated copper ribbon on the inside of the can (Mistal et al. 2000, Pol et al. 2017). When a cockroach contacted the insulated copper ribbon (1st electrode) walking on the inside wall of the electrical trap and was still in contact with the metal wall (2nd electrode), it received an electric shock and fell into the bottom of the trap, unable to escape. On completion of a bioassay after 15 h, GCRs were classified as responders if they were at the bottom of a trap. After each replicate, the arena and the traps were cleaned with Fisher brand Sparkleen® Laboratory detergent (2 g; Fisher Scientific Co., Pittsburgh, PA 15219, USA) in a moist paper towel. The two electrical traps were placed opposite to one another (180˚) in the arena 5 cm from the wall (Mistal et al. 2000, Pol et al. 2017).

Treatment and control stimuli were randomly assigned to each of the two traps. Treatment stimuli were tested in various combinations against each other, water, or an unbaited (empty) control. The desired amount of each stimulus was placed in the Petri dishes inside the electrical traps. Liquid test stimuli were quantified in a 4-ml test tube, or when smaller amounts were needed, with a pipette. Stimuli were poured directly into the

Petri dish. There were eightarenas deployed each bioassay day, allowing us to concurrently run replicates of multiple experiments under the same conditions and thus to compare the relative attractiveness of test stimuli across experiments.

For each experimental replicate, 12 GCR males (total) were collected from rearing cages in two plastic tubes (12.0 cm long × 3.0 cm diameter) containing a paper towel (1.5 cm × 4.0 cm) and a dry cotton ball. The tubes were closed with screw-on lids. Prior to bioassays, males were starved for 48 h unless otherwise stated to enhance their propensity to respond to food volatiles.

Experimental replicates were started at the onset of the scotophase (set to 15:00 h). The two tubes containing six GCRs each were placed at the centre of the arena and the cork stoppers were removed so that males could exit on their own accord. At this stage, dead males were replaced to ensure the same number of bioassay insects for each replicate. To facilitate optimal (dark) foraging conditions, a Plexiglas® lid was placed on each arena and covered with a dark plastic sheet, and all external lights were turned off. There were eight arenas set up in the same room. The temperature in that room was maintained at 25 ± 2 °C for the entire duration of each bioassay, lasting 15 h.h.

3.3.3. Composition and preparation of stimuli

Experimental test stimuli consisted of (1) a 3-component composition (3CC) [dry malt extract (DME), water, Brewer's yeast], (2) a DME/water mixture, (3) DME, and (4) various commercial cockroach baits.

A stock solution of the 3CC was prepared from 15 g of Golden light dry malt extract (DME) (Briess Malt & Ingredients Co., Chilton, WI 53014, USA), 150 ml of water (Burnaby Municipal tap water), and 0.15 g of Belgian saison-style Brewer's yeast (Lallemand Inc., Montreal, QC H1W 2N8, Canada). This strain of yeast was chosen for its high attenuation (sugar metabolism) and characteristic aroma (Gallone et al. 2016). The stock solution was kept in a 250-ml Erlenmeyer flask and typically allowed to ferment for 24 h prior to testing aliquots (1.5 and 4 ml) in bioassay replicates. The DME/water mixture was tested at 1.5- or 4-ml aliquots, and the DME on its own was tested at 1.5 g.

To eliminate the effect of yeast contaminants or other spoilage organisms in the DME or the tap water on the 3CC attractiveness, the DME/water mixture was boiled for 5 min, then cooled to room temperature, covered with a piece of aluminum foil and "aged" for 24 h prior to testing aliquots (1.5 and 4 ml) in experimental replicates. In specific experiments (see below), the boiled DME/water mixture was cooled, Brewer's yeast were added and allowed to metabolize for 6, 12, 20 or 24 h prior to testing aliquots (1.5 or 4 ml) in experimental replicates.

All commercial baits tested were removed from purchased bait stations (Combat-Insect Control Systems, Scottsdale, AZ 85254, USA). Commercial baits comprised (i) Combat® Source Kill Max Small Roach Bait ["Combat Small Roach" (CSR), 1.2 g], (ii) Combat® Source Kill Max Large Roach ["Combat Large Roach" (CLR), 2.0 g], and (iii) Combat® Source Kill Max Roach Gel ["Combat Roach Gel" (CRG), 1.5 g]. Each Combat bait was placed in a Petri dish (5.0 cm diam × 1.0 cm high) and covered with an aluminum-screen lid to prevent GCR feeding and spread of insecticidal ingredients in the laboratory bioassay arena. The control stimulus was an empty Petri dish unless otherwise stated.

3.3.4. Specific Experiments

Attractiveness of the 3-Component-Composition (3CC)

Laboratory experiments 1-3 (Table 1) tested the effect of the unboiled 3CC (4 ml) as a trap bait *vs* that of an unbaited control on captures of GCR males (Exp. 1, n = 8), females (Exp. 2, n = 12) and nymphs (Exp. 3, n = 12).

Effect of yeast on the 3CC attractiveness

With evidence that the 3CC attracted GCRs irrespective of gender and developmental stage (see Results), experiments 4-9 (Table 1) were designed to isolate the contributing effect of Brewer's yeast on the 3CC attractiveness. To that end, parallel experiments 4-5 tested the 3CC (Exp. 4) and the DME/water mixture (lacking Brewer's yeast) (Exp. 5) each vs a water control stimulus.

To address the possibility that the DME may contain some yeast or other spoilage organisms that could substitute for the absence of Brewer's yeast, the DME/water mixture was boiled 5 min, and then cooled to, and kept at, room temperature for 24 h before it was tested vs a water control stimulus (Exp. 6; n = 8). In parallel experiment 7

(n = 8), the DME/water mixture was also boiled 5 min but Brewer's yeast were added to the cooled mixture and allowed to ferment for 24 h before the solution was tested vs a water control stimulus.

To address the further possibility that yeast contaminants in the DME and Brewer's yeast added to the DME/water mixture may have additive or synergistic effects on the attractiveness of the composition, parallel experiments 8 (n = 12) and 9 (n = 12) tested the 3CC with or without boiling the DME/water mixture prior to adding Brewer's yeast. Both experiments were conducted 24 h after yeast were was added.

Effect of fermentation period on 3CC attractiveness

To determine whether the length of the fermentation period affects the attractiveness of the 3CC, the DME/water mixture was boiled and cooled before Brewer's yeast were added and allowed to ferment (i) for 6 h and 20 h in parallel experiments 10 (n = 13) and 11 (n = 13), and (ii) for 12 h and 24 h in parallel experiments 12 (n = 13) and 13 (n = 12) prior to testing GCR attraction (Table 1). In each of experiments 10–13, water served as the control stimulus.

As boiling the DME/water mixture prior to adding Brewer's yeast did not alter the 3CC attractiveness to GCRs (see Results), and as the 3CC was most attractive to GCRs after 24 h of fermentation (see Results), it was warranted to gauge the longevity of the 3CC attractiveness. Therefore, parallel experiments 14 (n = 12) and 15 (n = 12) (Table 1) tested 3CC attractiveness after 24 h and 120 h, respectively, of fermentation. In each of experiments 14 and 15, water served as the control stimulus in the paired traps.

Comparative attractiveness of the 3CC and commercial cockroach baits

The 3CC strongly attracted GCRs (see Results), invoking interest to compare its effectiveness as a trap bait to that of commercial Combat baits (CSR, CLR, CRG; see above). Therefore, parallel experiments 16-19 (Table 1) tested attraction of GCRs to traps baited with the (i) unboiled 3CC (Exp. 16, n = 12), (ii) CSR (Exp. 17, n = 12), (iii) CLR (Exp. 18, n = 12) and (iv) CRG (Exp. 19, n = 12). In each of experiments 16-19, paired control traps were left unbaited.

Because the 3CC seemed as attractive to GCRs as the Combat baits (see Results), three sets of follow-up experiments (Table 1) tested the unboiled 3CC vs CSR [Exp. 20

(n = 9) with males, Exp. 21 (n = 21) with females, Exp. 22 (n = 22) with nymphs], the unboiled 3CC vs CLR [Exp. 23 (n = 8) with males, Exp. 24 (n = 12) with females, Exp. 25 (n = 12) with nymphs] and the unboiled 3CC vs CRG [Exp. 26 (n = 8) with males, Exp. 27 (n = 12) with females, Exp. 28 (n = 12) with nymphs].

Comparative effectiveness of trap baits (3CC, DME, CLR) on GCR captures in field experiments

Two sets of two parallel experiments each (Table 1, Experiments 31-32; 33-34) were run in GCR-infested kitchens in Richmond and in Vancouver from 10 to 22 August 2016. Experimental replicates consisted of paired unscented sticky traps (Bell Laboratories, Inc. Madison, WI 53704, USA) (sticky surface 15.9 cm □ 10.8 cm) placed on the floors of two infested kitchens, with 25- cm spacing between paired traps and > 50 cm spacing between pairs. Each trap was folded into a rectangular, open-ended prism, with the adhesive surface on the inside. Within each trap pair, one trap by random assignment was baited with the test stimulus (1.5 ml of the 3CC, 1.5 g DME, or 2 g CLR) that was placed into a Petri dish (4.0 cm diam □ 1.0 cm high) in the center of the trap. The control trap in each pair contained an empty Petri dish. After 48 h or 72 h, captures of GCRs in traps were recorded, and traps and baits were replaced with new ones.

Data analyses

All data were analyzed with SAS statistical software version 9.4. Laboratory data were analyzed using a binary logistic regression model with a Firth bias correction to compare mean proportions of responders between test stimuli in each experiment. Type 3 effects for fixed-effect factors (Exps. 1-3, 4-5, 6-7, 8-9, 10-11, 12-13, 14-15, 16-19, 20-22, 23-25, 26-28, 29-30, 31-32, and 33-34) were used to determine mean differences in the proportion of responders to treatment stimuli between experiments. Post hoc tests with Tukey-Kramer adjustment were used to locate differences in mean proportions of responders to treatment stimuli between pairs of experiments.

Field data were analyzed with a binary logistic model using the GLIMMIX procedure. Data were analyzed with a paired design considering the effects of location and experiment as random factors.).

3.4. Results

Attractiveness of the 3-Component Composition (3CC)

Traps baited with the 3CC captured significantly more GCR males, females and nymphs than unbaited control traps (Exp. 1: males: $z = 3.96$, $p < 0.0001$; females: $z = 4.92$, $p < 0.0001$; nymphs: $z = 2.85$, $p = 0.0043$; Fig. 1). There was no difference in the proportions of males, females and nymphs captured in 3CC-baited traps.

Effect of yeast on the 3CC attractiveness

Traps baited with the 3CC (Exp. 4), or just the DME/water mixture (Exp. 5), captured significantly more GCR males than traps containing a water control stimulus (Exp. 4: $z = 6.42$, $p < 0.0001$; Exp. 5: $z = 6.86$, $p < 0.0001$; Fig. 2). There was no difference in the proportions of males captured in 3CC-baited traps or DME/water-baited traps (Tukey-Kramer, $z = - 0.72$, $p = 1.00$). These data indicate that yeast contaminants in the DME/water mixture increased its attractiveness.

Traps baited with the boiled DME/water mixture (lacking living yeast) as well as traps baited with a boiled DME/water mixture [(DME + W) (boiled)] receiving Brewer's yeast after cooling it, captured significantly more GCR males than traps containing a water control stimulus (Exp. 6: $z = 2.93$, $p = 0.0034$; Exp. 7: $z = 5.05$, $p < 0.0001$; Fig. 2). There was no significant difference in the proportions of captures in treatment traps of experiments 6 and 7 (Tukey-Kramer, $z = - 1.84$, $p = 0.99$) but the bait with Brewer's seemed more effective.

Traps baited with the unboiled 3CC (Exp. 8), or with the boiled DME/water mixture receiving Brewer's yeast after cooling it (Exp. 9), captured significantly more GCR males than traps containing a water control stimulus (Exp. 8: $z = 6.62$, $p< 0.0001$; Exp. 9: $z = 6.11$, $p < 0.0001$; Fig. 2). There was no difference in the proportion of captures in treatment traps of experiments 8 and 9 (Tukey-Kramer, $z = 0.58$, $p = 0.57$). These data combined indicate that there was no additive or synergistic effect between the DME yeast contaminants and Brewer's yeast.

Effect of fermentation period on attractiveness of the 3CC

Traps baited with the boiled DME/water mixture to which – once cooled – Brewer's yeast were added and allowed to metabolize for (i) 6 h and 20 h (parallel Exps. 10, 11), or (ii)

12 h and 24 h (parallel Exps. 12, 13) all attracted significantly greater proportions of males than traps containing a water control stimulus (Exp. 10: $z = 3.19$, $p = 0.0014$; Exp. 11: $z = 5.72$, $p < 0.0001$; Exp. 12: $z = 4.37$, $p < 0.0001$; Exp. 13: $z = 5.95$, $p < 0.0001$; Fig. 3). For each pair of the two parallel experiments, there was no significant difference in the proportion of males captured in treatment traps (Tukey-Kramer: Exp. 10 vs. 11: $z = -2.13$, $p = 0.95$; Exp. 12 vs. 13: $z = -1.60$, $p = 1.00$) but in each pair, baits with longer fermentation times seemed more attractive. Traps baited with the 3CC that was allowed to ferment for 24 h (Exp. 14) or 120 h (Exp. 15) prior to testing, all captured significantly greater proportions of GCR males than traps containing a water control stimulus (Exp. 14: $z = 6.76$, $p < 0.0001$; Exp. 15: $z = 6.08$, $p < 0.0001$; Fig. 3). Treatment traps in experiments 14 and 15 captured similar proportions of males (Tukey-Kramer, $z = 0.74$; $p = 1.00$).

Data of experiments 10-15 in combination suggest that the length of the fermentation period affects the attractiveness of the bait and that the attractive effect is persistent for at least 120 h.

Comparative attractiveness of the 3CC and commercial cockroach baits

Traps baited with the 3CC (Exp. 16), CSR (Exp. 17), CLR (Exp. 18), or CRG (Exp. 19), all captured significantly greater proportions of GCR males than unbaited control traps (Exp. 16: $z = 7.55$, $p < 0.0001$; Exp. 17: $z = 7.19$, $p < 0.0001$; Exp. 18: $z = 7.87$, $p < 0.0001$; Exp. 19: $z = 7.72$, $p < 0.0001$; Fig. 4). Proportions of trap captures in treatment traps of experiments 16-19 did not differ.

When the 3CC was tested vs CSR, the proportions of captures in 3CC- and CSR-baited traps did not differ for GCR males (Exp. 20), females (Exp. 21) and nymphs (Exp. 22) (Exp. 20: $z = -1.10$, $p = 0.27$; Exp. 21: $z = -0.37$, $p = 0.71$; Exp. 22: $z = -0.68$, $p = 0.50$; Fig. 4). CCE-baited trap captured similar proportions of males, females and nymphs.

When the 3CC was tested vs CLR, the proportion of captures in 3CC- and CLR-baited traps did not differ for males (Exp. 23), females (Exp. 24) and nymphs (Exp. 25) (Exp. 23: $z = 0.75$, $p = 0.45$; Exp. 24: $z = 0.70$, $p = 0.49$; Exp. 25: $z = 0.69$, $p = 0.49$; Fig. 4). CCE-baited traps captured similar proportions of males, females and nymphs.

When the 3CC was tested vs CRG, the proportions of GCR captures in 3CC- and CRG-baited traps did not differ for males (Exp. 26), females (Exp. 27) and nymphs (Exp. 28) (Exp. 26: $z = 0.35$, $p = 0.73$; Exp. 27: $z = -1.11$, $p = 0.27$; Exp. 28: $z = -0.12$, $p = 0.91$; Fig. 4). CCE-baited traps captured similar proportions of males, females and nymphs.

When 3CC was tested vs CRG (Exp. 29), 3CC-baited traps captured significantly greater proportions of males than CRG-baited traps ($z = -2.72$, $p = 0.0065$). Conversely, the proportions of males captured in DME- and CRG-baited traps did not differ (Exp. 30: $z = 1.36$, $p = 0.17$; Fig. 4). CRG-baited traps captured similar proportions of males in both experiments (Tukey-Kramer, $z = -2.89$, $p = 0.47$).

Comparative effectiveness of trap baits (3CC, DME, CLR) on GCR captures in field experiments

In parallel field experiments 31 and 32, 3CC-baited traps (Exp. 31) and CLR-baited traps (Exp. 32) captured significantly greater proportions of GCRs than unbaited control traps (Exp. 31: $z = 3.88$, $p = 0.0001$; Exp. 32: $z = 4.69$, $p < 0.0001$; Fig. 5). Proportions of treatment trap captures did not differ between the two experiments (Tukey-Kramer, $z = -1.90$, $p = 0.23$).

In parallel field experiments 33 and 34, DME-baited traps (Exp. 33) and CLR-baited traps (Exp. 34), captured significantly greater proportions of GCRs than unbaited control traps (Exp. 33: $z = 2.89$, $p = 0.0039$; Exp. 34: $z = 2.38$, $p = 0.02$; Fig. 5). Proportions of treatment trap captures did not differ between the two experiments (Tukey-Kramer, $z = 0.68$, $p = 0.91$).

3.5. Discussion

Our data show that (1) a 3-component composition (3CC) [dry malt extract (DME) + Water + Brewer's yeast] attracts GCRs irrespective of their developmental stage and gender; (2) Brewer's yeast and yeast contaminants (spoiler organisms) each seem to contribute to the 3CC attractiveness but there is no interactive effect between them; (3) the 3CC attracts GCRs as effectively as each of three commercial cockroach baits; and (4) the 3CC, or just the DME, each is as effective as a commercial cockroach bait in field trapping experiments.

The unboiled 3CC significantly attracted GCR nymphs, females and males (Fig. 1; Exps. 1-3) but the presence or absence of Brewer's yeast in the unboiled composition had no immediately obvious effect on its attractiveness (Fig. 2; Exps. 4, 5). This was unexpected because we assumed that actively metabolizing Brewer's yeast produce the key semiochemicals, such as ethanol (Karimifar et al. 2011, Bokulich et al. 2012), that attract GCRs. We then hypothesized that yeast contaminants or other spoilage organisms in the DME or the water may have substituted for the absence of Brewer's yeast in the composition. To test this hypothesis, we (i) added water to the DME, (ii) boiled the mixture, (iii) allowed the mixture to cool (20 ºC), and then (iv) bioassayed it with and without prior addition of Brewer's yeast for attraction of GCRs.

Even though the mixture with or without Brewer's yeast significantly attracted GCRs (Fig. 2; Exps. 6,7), the mixture with Brewer's yeast seemed more effective (see also parallel experiments 4 and 5 for comparison), suggesting that yeast contaminants or other spoilage organisms in the mixture had indeed been killed, or their metabolic activity been suppressed, through boiling and that the addition of Brewer's yeast compensated for their death or metabolic inactivity. Conversely, these results also suggest that living yeast contaminants in the mixture produce semiochemicals that attract GCRs. In retrospect, these results could have been expected. The boiling step is the part of the beer brewing process that ensures the elimination of all those unfavorable yeasts and microbes that otherwise would metabolize DME nutrients and in the process alter the desired flavor and taste of the future beverage (Bokulich et al. 2012).

The 3CCs with or without the boiling step prior to adding Brewer's yeast contained dead and live yeast contaminants, respectively, but equally attracted GCRs (Fig. 2; Exps. 8,9). These results indicate that there was no additive or synergistic effect between the semiochemicals produced by living yeast contaminants and Brewer's yeast. Therefore, even if all these yeasts and bacteria were to belong to different taxonomic groups, they must have produced volatile blends that contained one or more of the same key semiochemicals that attract GCRs. Such overlap in odor profiles produced by diverse taxa of yeast has previously been reported. For example, both isoamyl acetate and ethyl acetate are produced by each of three species of yeast: Cyberlindnera saturnus (Williopsis saturnus), Hanseniaspora uvarum and Wickerhamomyces subpeliculosus (Basso et al. 2016).

To understand the evolution and longevity of attractiveness inherently linked to a composition with metabolizing yeast, it was important to determine both (i) the duration of fermentation required for the composition to become attractive, and (ii) the attraction duration of an optimally attractive composition. A composition with extended lag time to become attractive or a short-lived attractive effect would not meet the criteria of a trap bait or bait station in GCR abatement programs. Our data show that over the course of 6 to 24 h of fermentation, the 3CC becomes increasingly more attractive (Fig. 3; Exps. 10-13), attains peak attractiveness at ~24 h (Fig. 3; 12,13), and then remains optimally attractive for at least 120 h (Fig. 3; Exps. 14,15). The observed increase in attractiveness could be attributed to rapid growth of the yeast population (Lodolo et al. 2008) that utilizes the rich DME nutrient source and, in the process, produces ever increasing amounts of the semiochemicals that attract GCRs. It is yet to be determined whether after 120 h of fermentation the Brewer's yeast reduce metabolic activity or change metabolic pathways, thus possibly rendering the composition less attractive.

The 3CC exhibits a level of attractiveness to GCRs comparable to that of commercial cockroach control technologies [Combat Small Roach (CSR), Combat Large Roach (CLR), and Combat Roach Gel (CRG)]. In four parallel experiments, the 3CC, CSR, CLR, and CRG were equally effective in attracting GCR males (Fig. 4; Exps. 16-19). However, because the food preferences of GCRs vary in accordance with their stage of development (Kunkel 1966), sex and reproductive status (Durbin and Cochrane 1985), it seemed prudent to also compare the responses of GCR nymphs and females to the 3CC and Combat Roach technologies. For these experiments (Fig. 4; 20-28), we changed the experimental design in that we tested two treatment stimuli, instead of one treatment and one control stimulus, in each experimental replicate. Testing the responses of GCR nymphs, females and males in each of three parallel experiments to the same set of paired treatment stimuli (Exps. 20-22: 3CC vs CSR; Exps. 23-25: 3CC vs CLR; Exps. 26-28: 3CC vs CRG) allowed us to most rigorously assess the relative attractiveness of the 3CC to all segments of GCR populations. Trap capture data of GCR nymphs, females and males (Fig. 4; Exps. 20-28) reveal that the 3CC was indeed competitive with any of the three Combat Roach products. Moreover, males were generally captured in greater portions than females or nymphs, supporting previous findings that males have the greatest propensity to forage (Smith and Appel 2008).

Despite the convincing efficacy of the “wet” 3CC, a dry bait (e.g., DME) that would be stored and deployed without water remains desirable. It simply alleviates many of the challenges associated with “wet” baits, including the longevity of the bait and the preservation of lethal agents therein. Therefore, we wanted to rigorously field-test the bait potential of the dry DME, and thus tested it and the 3CC in parallel, each versus the CRG (Fig. 5). We used CLR as the commercial bait because it seemed to have performed slightly better than the CRG and CSR in preceding laboratory experiments. In parallel field experiments 31 and 32, the 3CC and CLR baits were each more attractive to GCRs than unbaited control traps (Fig. 5). Similarly, in parallel experiments 33 and 34, the DME and CLR were each more attractive to GCRs than unbaited control traps (Fig. 5). These results combined indicate that both the 3CC and the DME could become commercial baits, with the DME likely being less challenging to develop commercially.

While all baits tested in field experiments had a significant effect on GCR captures, the baits merely doubled captures relative to those in unbaited control traps. This seemingly modest effect is attributed to alternative food sources that foraging GCRs could access in the kitchens where the experiments were run. As there will often be competing food sources in GCR-infested premises, baited retainer traps on their own, although useful, are not sufficient for effective GCR control and are more suited for monitoring populations (Ballard and Gold 1984, Miller et al. 2000). Conversely, poisonous baits have been implemented successfully in various GCR abatement programs and present an effective treatment option for the foreseeable future (Mallis 2011). The lethal agents in these poisonous baits are not necessarily chemical insecticides. They can instead be biological control agents such as the fungi (Zurek et al. 2002) and entomopathogenic nematodes (Maketon et al. 2010).

Future studies will investigate lethal biocontrol agents that can be added to the DME or the 3CC, and explore the efficacy of such lethal baits for GCR control.

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Table 3.1 *Research objectives (O), number of replicates (*n*), and stimuli tested for the responses of German cockroaches in laboratory still-air arena experiments 1-30 (Exps. 1-30) and in field experiments 31-34.*

Exp.[1]	n[2]	Age/Sex	Stimulus 1	Stimulus 2
O1: Determine the attractiveness of the 3CC to juvenile and adult GCRs				
1	8	Males	DME[3] + W[4] + BY[5] (1.5 ml)	Unbaited
2	12	Females	DME + W + Y (1.5 ml)	Unbaited
3	12	Nymphs	DME + W + Y (1.5 ml)	Unbaited
O2: Study the effect(s) of Brewer's yeasts and yeast contaminants on 3CC attractiveness				
4	12	Males	DME + W (4 ml)	Water[4] (4 ml)
5	12	Males	DME + W + BY (4 ml)	Water (4 ml)
6	8	Males	[(DME + W) (boiled)]	Water (4 ml)
7	8	Males	[(DME + W) (boiled)] (4 ml) + BY (4 ml)	Water (4 ml)
8	12	Males	[DME + W] (4 ml)	Water (4 ml)
9	12	Males	[(DME + W) (boiled)] + BY (4 ml)	Water (4 ml)
O3: Investigate the effect of fermentation period (6-120 h) on 3CC attractiveness				
10	13	Males	[(DME + W) (boiled)] + BY (4 ml) Fermentation: 6 h	Water (4 ml)
11	13	Males	[(DME + W) (boiled)] + BY (4 ml) Fermentation: 20 h	Water (4 ml)
12	13	Males	[(DME + W) (boiled)] + BY (4 ml) Fermentation: 12 h	Water (4 ml)
13	12	Males	[(DME + W) (boiled)] + BY (4 ml) Fermentation: 24 h	Water (4 ml)
14	12	Males	[(DME + W) (boiled)] + BY (4 ml) Fermentation: 24 h	Water (4 ml)
15	12	Males	[(DME + W) (boiled)] + BY (4 ml) Fermentation: 120 h	Water (4 ml)
O4: Compare the 3CC attractiveness to that of commercial GCR baits				
16	12	Males	DME + W + BY (1.5 ml)	Unbaited
17	12	Males	Combat Small Roach (1.2 g)	Unbaited
18	12	Males	Combat Large Roach (2 g)	Unbaited
19	12	Males	Combat Roach Gel (1.5 g)	Unbaited
20	9	Males	DME + W + BY (1.5 ml)	Combat Small Roach (1.2 g)
21	13	Females	DME + W + BY (1.5 ml)	Combat Small Roach (1.2 g)
22	13	Nymphs	DME + W + BY (1.5 ml)	Combat Small Roach (1.2 g)

Table 3.1 continued…

23	8	Males	DME + W + BY (1.5 ml)	Combat Large Roach (2 g)
24	12	Females	DME + W + BY (1.5 ml)	Combat Large Roach (2 g)
25	12	Nymphs	DME + W + BY (1.5 ml)	Combat Large Roach (2 g)
26	8	Males	DME + W + BY (1.5 ml)	Combat Roach Gel (1.5 g)
27	12	Females	DME + W + BY (1.5 ml)	Combat Roach Gel (1.5 g)
28	12	Nymphs	DME + W + BY (1.5 ml)	Combat Roach Gel (1.5 g)
29	11	Males	DME + W + BY (1.5 ml)	Combat Roach Gel (1.5 g)
30	11	Males	DME (1.5 g)	Combat Roach Gel (1.5 g)
O6: Compare the efficacy of the 3CC, DME and a commercial bait for field trapping GCRs				
31	49	GCRs[6]	DME + W + BY (1.5 ml)	Unbaited
32	51	GCRs	Combat Large Roach (2 g)	Unbaited
33	58	GCRs	DME (1.5 g)	Unbaited
34	58	GCRs	Combat Large Roach (2 g)	Unbaited

[1]Experiments 1-3, 4-5, 6-7, 8-9, 10-11, 12-13, 14-15, 16-19, 20-22, 23-25, 26-28, 29-30, 31-32, and 33-34 were conducted in parallel.

[2]Each laboratory replicate tested 12 48-h starved cockroaches of a particular age or sex

[3]Golden light dry malt extract (DME); Briess Malt & Ingredients Co., Chilton, WI, USA.

[4]Burnaby Municipal tap water (W).

[5]Belgian saison-style Brewer's yeast (BY), Lallemand Inc., Montreal, QC, Canada.

[6]Males, females and nymphs of German cockroaches (GCRs)

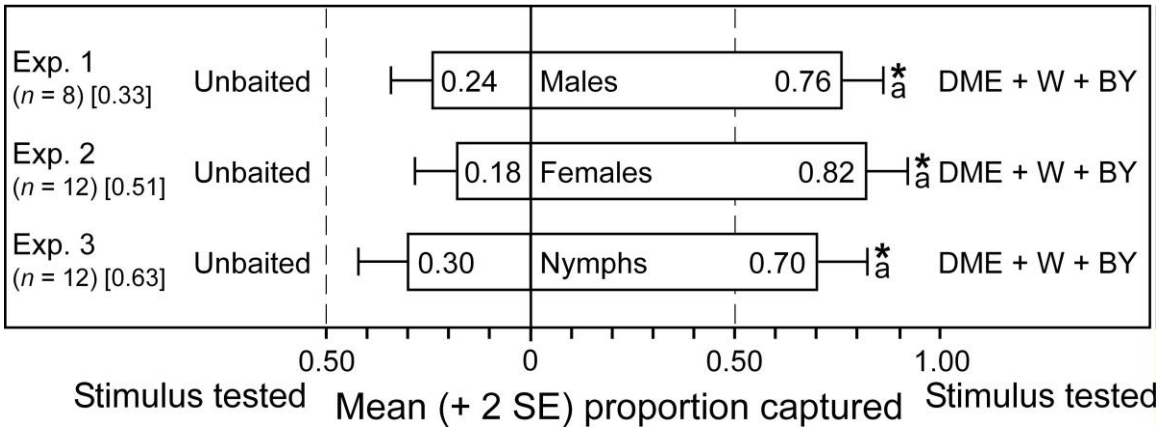

Figure 3.1 *Back-transformed mean (+2 SE) proportions of Blattella germanica males, females and nymphs captured in arena experiments in paired traps baited with a composition of dry malt extract (DME), water (W) and Brewer's yeast (BY) (fermented for 24 h) or left unbaited (Table 1). For each experiment, an asterisk (*) on a bar denotes a significantly higher proportion of captures in treatment traps (in all experiments, $p < 0.001$). Proportions of captures in treatment traps in experiments 1-3 did not differ; n indicates the number of replicates; numbers in square brackets indicate the mean proportions of males, females or nymphs not captured in treatment or control traps.*

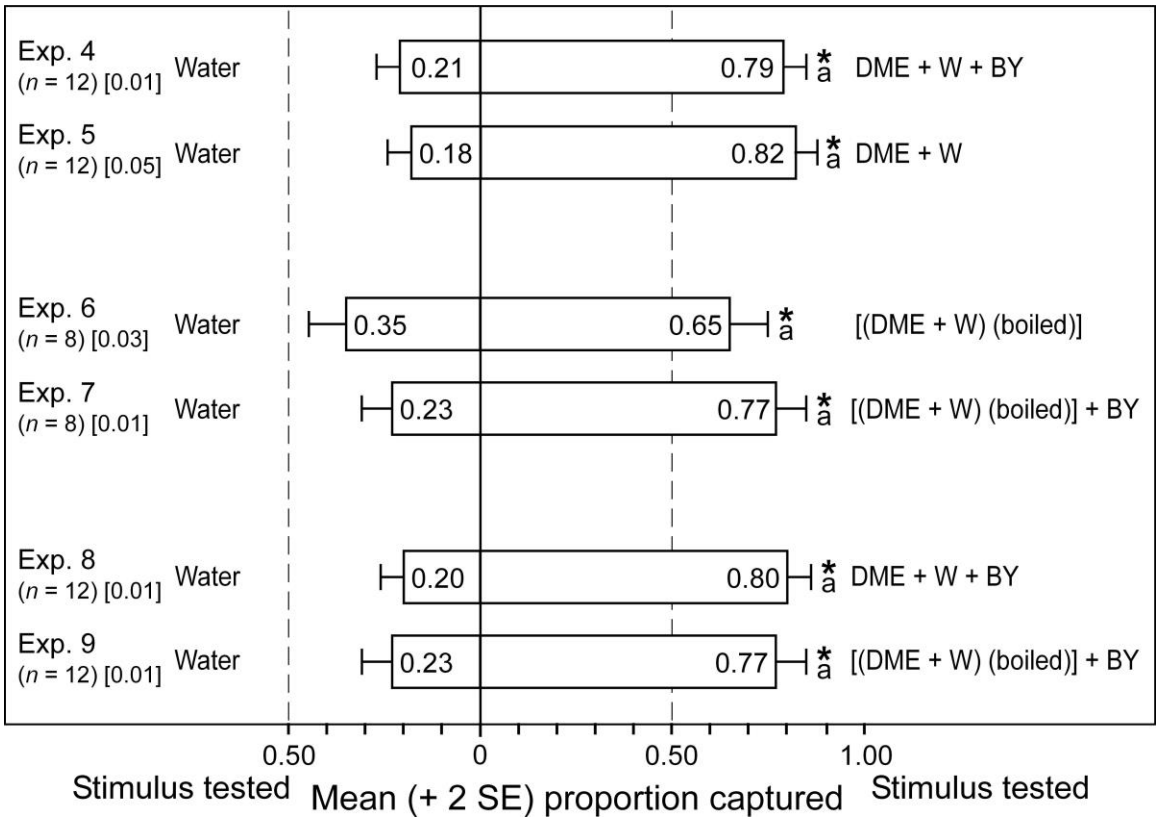

Figure 3.2 *Back-transformed mean (+ 2 SE) proportions of Blattella germanica males captured in arena experiments in paired traps baited with a composition of dry malt extract (DME) and water (W) that was boiled or not, and to which Brewer's yeast (BY) were added or not after the composition had cooled, with water serving as the control stimulus (Table 1). For each experiment, an asterisk on a bar denotes a significantly greater proportion of captures in treatment traps ($p < 0.005$). Proportions of captures in treatment traps did not differ among experiments 4 and 5, 6 and 7, and 8 and 9 (Tukey-Kramer, $p > 0.05$ for each pair); grouped experiments were run in parallel; n indicates the number of replicates; numbers in square brackets indicate the mean proportions of males not captured in treatment or control traps.*

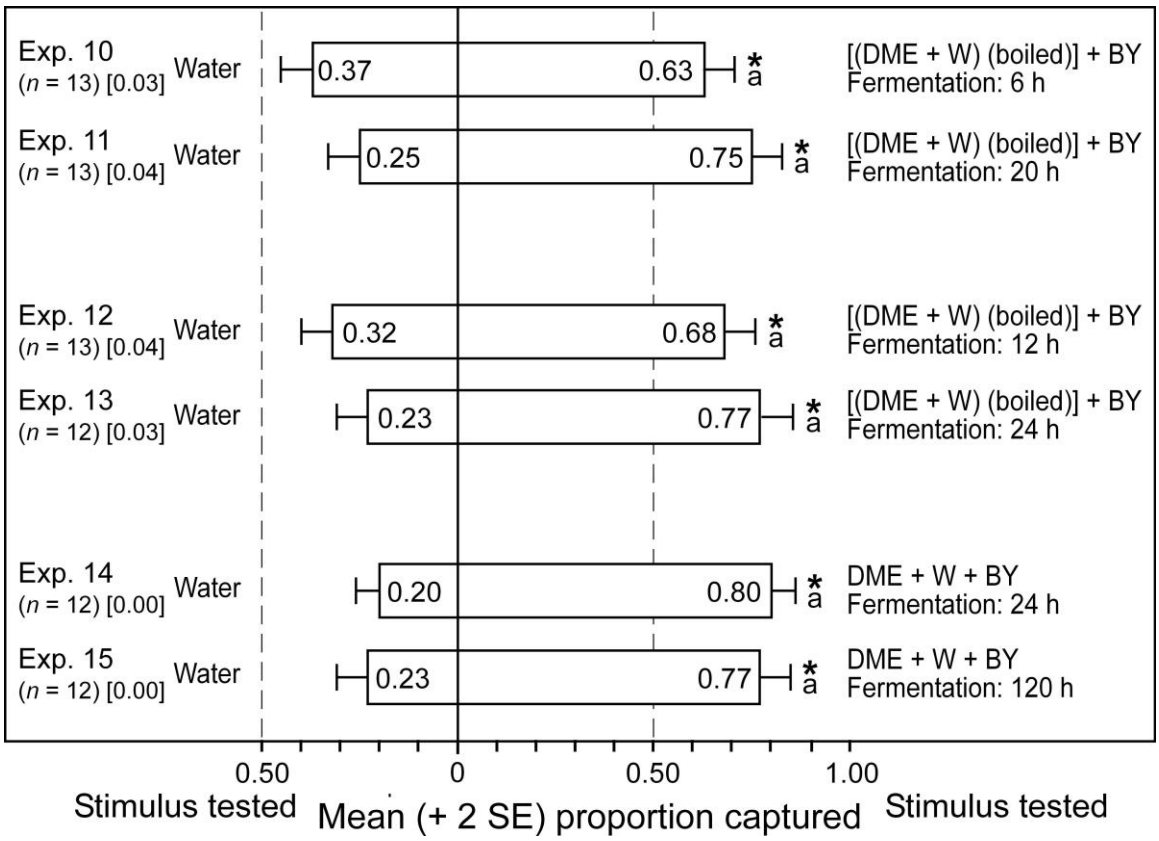

Figure 3.3 *Back-transformed mean (+ 2 SE) proportions of Blattella germanica males captured in two-choice arena experiments in traps baited with a composition of dry malt extract (DME), water (W) and Brewer's yeast (BY) that was allowed to ferment for 6-120 h prior to bioassays, with water serving as the control stimulus (Table 1). For each experiment, an asterisk on a bar denotes a significantly greater proportion of captures in treatment traps ($p < 0.005$). Proportions of captures in treatment traps did not differ among experiments 10 and 11, 12 and 13, and 14 and15 (Tukey-Kramer, $p > 0.05$ for each pair); grouped experiments were run in parallel; n indicates the number of replicates; numbers in square brackets indicate the mean proportions of males not captured in treatment or control traps.*

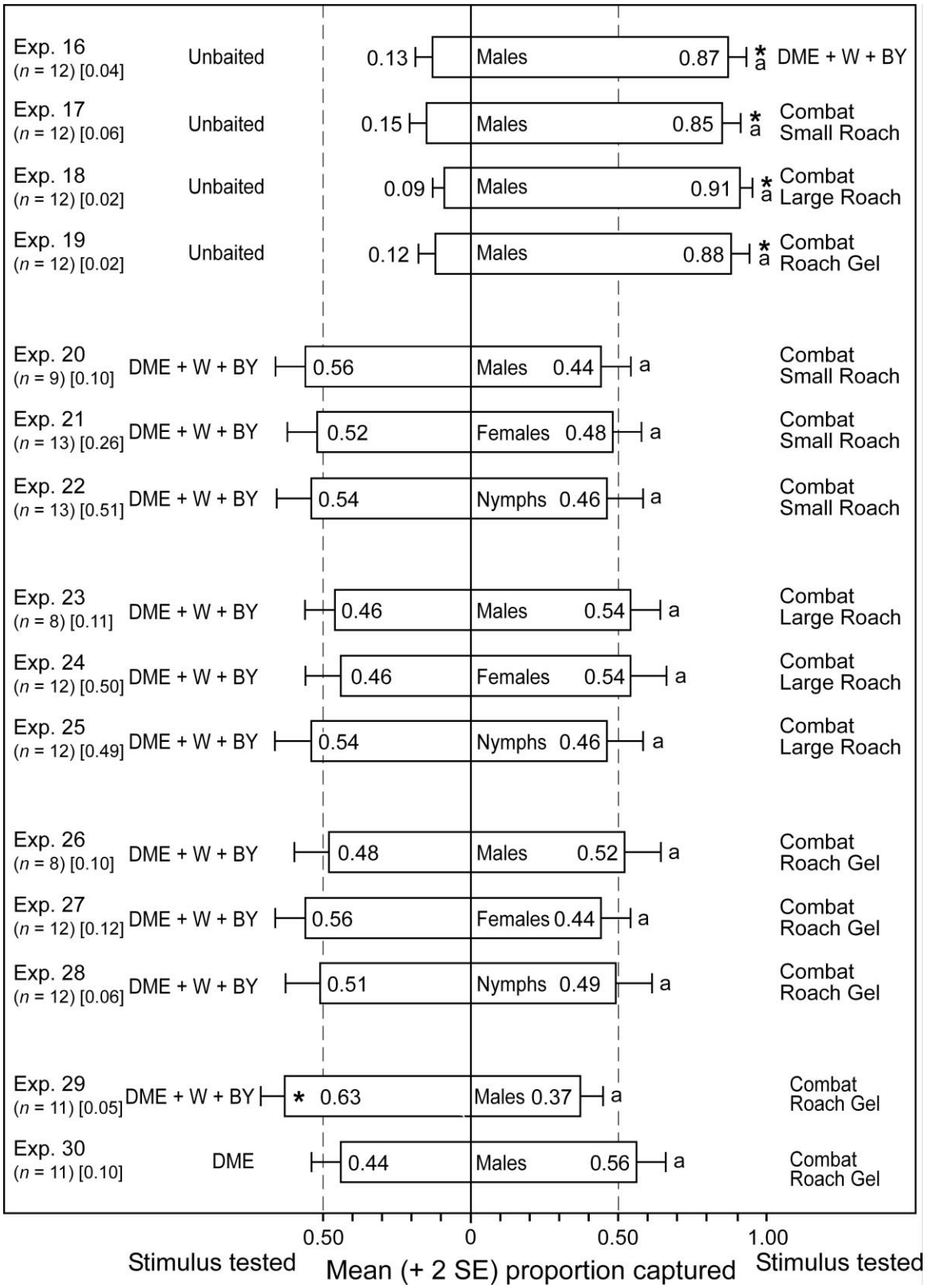

Figure 3.4 *Back-transformed mean (+ 2 SE) proportions of Blattella germanica captured in arena experiments in paired traps baited with (i) the 3-component composition of dry malt extract (DME), water (W)*

and Brewer's yeast (BY) (treatment traps) or left unbaited (Exps. 16-19) (Table 1), or (ii) the 3-component composition or a Combat commercial cockroach bait. For each experiment, an asterisk on a bar denotes a significantly greater proportion of B. germanica captured in traps baited with the respective stimulus ($p < 0.005$). Proportions of captures in the 3-component blend traps did not differ among experiments 16-19, 20-22, 23-25, 26-28 and 29-30. Grouped experiments were run in parallel; n indicates the number of replicates; numbers in square brackets indicate the mean proportions of males, females and nymphs not captured in treatment or control traps.

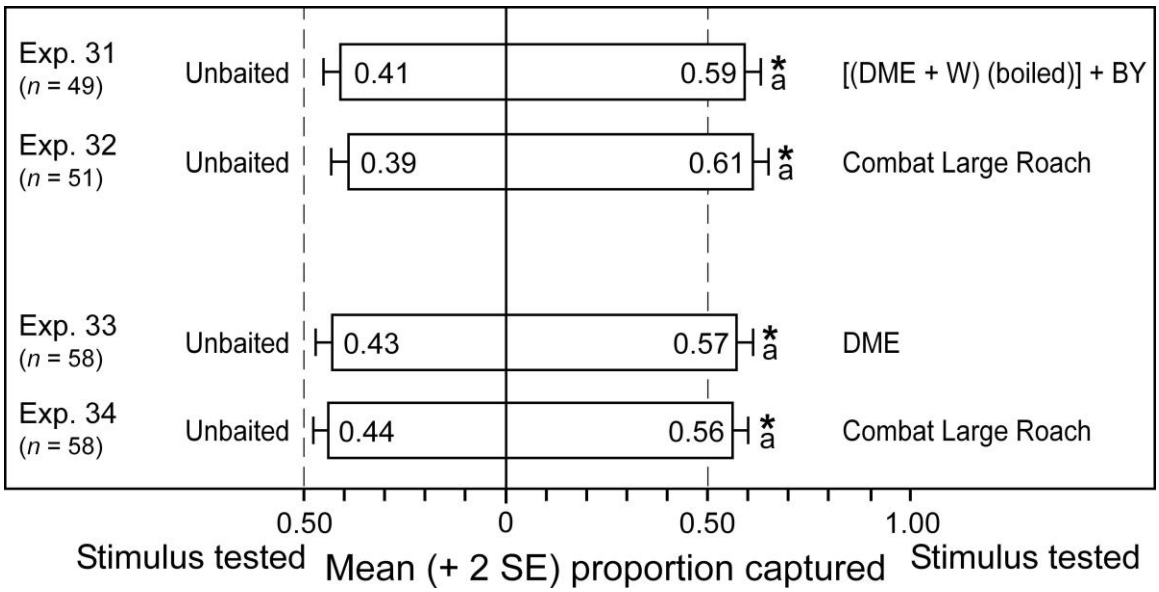

Figure 3.5 *Back-transformed mean (+ 2 SE) proportions of Blattella germanica individuals captured in field experiments in paired traps baited with the composition of dry malt extract (DME), water (W) and Brewer's yeast (BY), DME alone, or Combat large roach as test stimuli, with unbaited traps serving as corresponding controls (Table 1). For each experiment, an asterisk on a bar denotes a significantly greater proportion of captures in treatment traps ($p < 0.005$). Proportions of captures in treatment traps did not differ among experiments 31 and 32 (Tukey-Kramer, $p > 0.05$) and among experiments 33 and 34 (Tukey-Kramer, $p > 0.05$); grouped experiments were run in parallel; n indicates the number of replicates.*