Contributions to the foraging ecology of house flies, *Musca domestica* L.

by

Matthew V. Holl

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Name: **Matthew V. Holl**

Degree: **Master of Pest Management**

Title: ***Contributions to the foraging ecology of house flies, Musca domestica L.***

Examining Committee: **Chair:** Dr. Jenny Cory
Professor

Dr. Gerhard Gries
Senior Supervisor
Professor

Dr. Carl Lowenberger
Supervisor
Professor

Dr. Gail Anderson
Internal Examiner
Professor
School of Criminology

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Abstract

Attract-and-kill tactics for control of house flies (*Musca domestica*) often use foraging cues as attractants. To investigate foraging resources for indoor attraction of house flies, I tested the response of flies to various human foods and a floral resource. In two-choice laboratory bioassays, only dandelion flowers and dandelion honey attracted flies. Analytical attempts to capture the essential semiochemicals from these resources failed, highlighting the need to develop alternative approaches. Another potentially effective foraging cue is the “fly factor”, the phenomenon that food currently or previously fed on by flies attracts more flies than the same type of food kept inaccessible to flies. In two-choice laboratory bioassays, I demonstrate that the fly factor exists in house flies. Of the mechanisms tested potentially causing the fly factor, only fly feces and regurgitate attract flies. Attraction of flies to fly feces and regurgitate indicates that flies sense airborne semiochemicals emanating from these sources.

Keywords: *Musca domestica*, fly factor, foraging cues, semiochemical attractants, trapping

Dedication

This thesis is dedicated to my parents, Judi and Ken Holl. I can't imagine where I would be without their support and encouragement. To my brother, Chris. He inspires and impresses me more than he knows. And to my loving wife, Ashleigh. Her enthusiasm, reassurance, intelligence, passion, wit, perspective, and strength amaze me. I am so grateful to her.

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List of Acronyms

DCM	Dichloromethane
FDE	Flower distillate equivalent
FEE	Flower extract equivalent
FFs	Foraging flies
FHE	Flower hour equivalent (Ch. 2); fly hour equivalent (Ch. 3)
GDEH	Gram distillate equivalents of honey
GEEH	Gram extract equivalents of honey
GHEH	Gram hour equivalents of honey
RFs	Response flies
RH	Relative humidity
SFs	Stimulus flies

Glossary

Attract	To be motivated to physically approach something
Attractant	Something that causes an organism to be motivated to physically approach it
Cue	A stimulus that elicits a behaviour
Behaviour	The way an organism interacts with its environment
Foraging	Searching for food
Myophilous	Flowers that attract flies to nectar or pollen
Olfactory	Relating to odour
Oviposition	Relating to laying eggs
Pathogen	A biological agent that causes illness or disease
Regurgitate	Saliva and/or vomitus
Sapromyophilous	Flowers that attract flies by mimicking the odour of decaying substances
Semiochemical	Message-carrying chemical
Signal	A means of intentional information transfer
Symbiotic	Living together; for instance, one organism living within another
Tactic	Synonymous with method

Chapter 1.

Introduction

1.1. The House fly (*Musca domestica* L.)

The house fly, *Musca domestica* L. (Diptera: Muscidae) is a cosmopolitan insect that is well adapted to survive in and near human dwellings as well as livestock production facilities. House flies are an annoyance to both humans and animals and are important carriers of disease-causing pathogens.

1.1.1. Life History

House flies undergo complete metamorphosis with distinct egg, larval, pupal and adult stages. Adult house flies range in size from 5-12 mm in length and are non-metallic with a black and gray striped thorax. Adult house flies have a generalist diet which includes proteinous sources (e.g., feces or meat) that females require for egg development as well as carbohydrates (e.g., sugars) that females and males seek for sustenance and energy. Females typically mate 3-5 days post eclosion from the pupal case. Gravid females can lay more than 500 eggs in batches of about 100 over the course of their life, and usually lay their eggs in feces or other rotting organic matter (Moon & Meyer, 1985; Pedigo & Rice, 2009). Eggs typically hatch within 8-24 hours depending on environmental conditions, mostly temperature (Pedigo & Rice, 2009). After approximately 5-7 days of development in nutrient-rich feces or rotting organic matter, larvae pupate and develop into adults during another 5-7 days. The entire lifecycle typically takes 10-14 days depending on environmental conditions. House flies can overwinter in any life stage and may be present year round in indoor habitats such as livestock production facilities, restaurants and homes (Pedigo & Rice, 2009).

1.1.2. Distribution

House flies have cosmopolitan distribution, in part, because of their close association with humans. House flies can be found in urban settings such as homes, restaurants, and dumpsters (Moriya *et al.,* 1999; Butler *et al.,* 2010). Additionally, house flies are considered pest insects at landfills and livestock (poultry, cattle, etc.) production facilities (Goulson *et al.,* 1999; Mullen & Durden, 2009; Bahrndorff *et al.,* 2013).

1.2. House flies and disease

Mechanical vectors of food-borne pathogens often share specific traits that make them more likely to transport and spread pathogens to humans. These traits include synanthropy (close association with humans), endophily (gravitation towards buildings and the indoors), attraction to both filth and human food, migratory behaviour (oscillation between filth and human habitation), and the ability of natural (wild) populations to transport and/or harbour pathogens (Greenberg, 1971; Olsen, 1998; Olsen *et al.,* 2001). House flies are one of only a few filth flies that exhibit all of these traits, making them of particular concern for transmission of food-borne pathogens (Olsen, 1998). Wild house flies carrying traceable amounts of pathogenic bacteria including *Escherichia coli*, *Salmonella* spp*.* and *Campylobacter* spp*.* have been collected in a variety of settings closely linked with humans and human food production (Hald *et al.,* 2008; Butler *et al.,* 2010; Scallan *et al.,* 2011; Wang *et al.,* 2011; Gupta *et al.,* 2012; Barhndorff *et al.,* 2013).

1.2.1. Escherichia coli

Butler *et al.* (2010) tested flies captured near dumpsters and rear entrances of restaurants. Most of the bacteria isolated from these flies, including *E. coli* O157:H7, were potentially pathogenic or known human pathogens. House flies collected from animal houses, including pigpens, dog pounds, and turkey and poultry processing facilities also have tested positive for *E. coli* (Szalanski *et al.,* 2004; Förster *et al.,* 2007; Blaak *et al.,* 2015).

House flies were the mechanical vectors of an *E. coli* O157:H7 outbreak in a nursery school in rural Japan in 1996 (Moriya *et al.,* 1999). After eliminating food, water, drainage effluent, bird feces, and other substrates as potential sources of the bacteria, house flies in many school rooms were found to carry *E. coli* O157:H7. Molecular techniques revealed no discernable differences between the *E. coli* O157:H7 strain isolated from feces of infected children and the strain that house flies carried. Cross contamination of the nursery's dining area by house flies that traveled from a nearby cattle ranch was likely the means by which children became exposed to the pathogen. Transfer of *E. coli* from flies to sterile objects has been observed in laboratory experiments (De Jesús *et al.,* 2004).

1.2.2. Salmonella spp.

House flies have long been connected with typhoid outbreaks caused by *Salmonella typhi*. The connection was perceived to be so strong, especially during wartime, that house flies were briefly rebranded "typhoid flies" in the early 20th century (Cirillo, 2006). Today, *Salmonella* spp. is one of the most prevalent food-borne pathogens in the US, with over 1 million cases of non-typhoidal *Salmonella* spp. infections occurring annually (Scallan *et al.,* 2011). Of the patients hospitalized with a food-borne illness, 35% are infected with *Salmonella* spp., and 28% of deaths caused by food-borne pathogens are due to *Salmonella* spp. (Scallan *et al.,* 2011). House flies also are known carriers of various non-typhoidal *Salmonella* spp., especially in agricultural settings (Mian *et al.,* 2002; Ugbogu *et al.,* 2006; Wang *et al.,* 2011).

Greenberg *et al.* (1964) demonstrated experimentally the transmission of *S. typhimurium* from animal reservoirs to insect vectors to humans. They inoculated a healthy dog with *S. typhimurium,* collected its stool and fed it to uninfected house flies. The stool-exposed flies were then allowed to feed on atole (a Mexican beverage) before healthy volunteers drank the beverage. Although none of the volunteers showed symptoms of illness, 60% of their fecal samples tested positive for *S. typhimurium.* This experiment confirms that house flies can transport *S. typhimurium* from a fecal source in quantities sufficiently large to infect humans. The authors suspect that larger doses

would have resulted in the volunteers becoming ill. This finding provides strong evidence that house flies can transmit *S. typhimurium* from a fecal source to humans.

1.2.3. Campylobacter spp.

Campylobacter spp. is frequently present on raw chicken ready for human consumption (Christensen *et al.,* 2001). As a result, approximately 80% of *Campylobacter* spp. infections are food-borne and *Campylobacter* spp. is responsible for 15% of the food-borne illness hospitalizations in the US (Scallan *et al.,* 2011). House flies are known carriers of *Campylobacter* spp. and are considered important vectors (Rosef & Kapperud, 1983; Shane *et al.,* 1984; Hald *et al.,* 2004, 2008). Seasonal increases in house fly populations are highly correlated with human infections of *Campylobacter* spp. (Nichols, 2005). Efforts to discourage house flies from entering or remaining within homes may ultimately reduce the number of *Campylobacter* spp. infections in humans.

1.2.4. House flies as mechanical vectors

Most pathogens associated with house flies are thought to be spread mechanically (Levine & Levine 1991; Moriya *et al.,* 1999; Fasanella *et al.,* 2010). For instance, after house flies ingest *Campylobacter* spp. they exhibit an elevated immune response which inhibits the survival of the pathogen (Gill, 2014; Gill *et al.,* 2016). Therefore, house flies do not ingest, amplify, and increase the population *Campylobacter* spp. Instead, they inadvertently pick up pathogens on their tarsal pads, legs or body hairs, and then they inadvertently drop them wherever they happen to land (Nanzi *et al.,* 2005; Meerburg *et al.,* 2007; Fasanella *et al.,* 2010). Controlling house flies, often with vigilant trapping, has reduced the incidence of certain diseases (Cohen *et al.,* 1991; Moriya *et al.,* 1999; Christensen *et al.,* 2001; Bahrndorff *et al.,* 2013).

1.3. (Z)-9-Tricosene and its efficacy as a house fly attractant

Since the first evidence of pheromone-mediated sexual communication in house flies was presented, attempts have been made to elucidate the sex attractant pheromone and to coopt it for house fly control (Rogoff *et al.,* 1964). Carlson *et al.* (1971) discovered (Z)-9-tricosene as the main sex attractant pheromone component of female house flies and named it muscalure. Synthetic pheromone has since been used as bait for sticky panels, flypaper strips, sugar-toxicants, and electric grids (Carlson & Beroza, 1973; Rogoff *et al.,* 1973). Moreover, (Z)-9-tricosene has continuously been used on poultry, pig, and dairy farms to increase house fly capture on sugar-toxicants baits (Chapman *et al.,* 1998a, 1998b, 1999; Hanley *et al.* 2004; Butler & Mullens, 2010). Essentially all commercial house fly baits contain (Z)-9-tricosene as an attractant (Darbro *et al.,* 2005).

1.3.1. Lack of efficacy

In spite of its ubiquitous use, (Z)-9-tricosene has been found in multiple studies to be less effective than previously determined. While some efficacy trials do not show evidence of differential house fly captures in pheromone-baited or un-baited control traps (Mulla *et al.,* 1977; Butler *et al.,* 2007; Hanley *et al.,* 2009), other field studies report only marginal attractiveness of pheromone-baited traps (Morgan *et al.,* 1974; Carlson & Leibold, 1981; Hanley *et al.,* 2004).

Weak attraction of house fly males to (Z)-9-tricosene seems to indicate that (Z)-9-tricosene is only a component of the house fly sex pheromone (Uebel *et al.,* 1976; Adams & Holt, 1987) or that other sensory modalities play a role during sexual communication. Select compounds increase male mating activity near treated “pseudo flies” and reduce homosexual mating strikes (Uebel *et al.,* 1976; Rogoff *et al.,* 1980; Adams & Holt, 1987). These minor pheromone components appear to convey relevant information to prospective house fly mates and thus could be incorporated into house fly baits. However, to my knowledge no field studies have investigated the effect of these compounds in combination with (Z)-9-tricosene.

1.3.2. Biological significance

The biological significance of (Z)-9-tricosene is questionable. The amount of (Z)-9-tricosene found on laboratory-reared house flies is consistently greater than on field collected house flies, and some field populations have no detectable amounts of (Z)-9-tricosene (Noorman & den Otter, 2001; Darbro *et al.,* 2005). As populations lacking (Z)-9-tricosene exist and proliferate, males and females must be able to find one another via additional communication signals. The necessity and function of the house fly sex pheromone, both in the context of house fly biology and as a pest management tool, are uncertain.

1.4. Overview of house fly control methods with baits

House flies are pests in animal husbandry and near human habitation. In these two settings, different means for controlling adult house flies are needed. For instance, sugar toxicants that house flies consume and then quickly perish are commonly used in agricultural house fly control programs but are almost entirely banned near food preparation areas in homes and restaurants (Butler *et al.,* 2007; Carlson & Hogsette, 2007). The QuikStrike Fly Abatement Strip™ (Wellmark International, Schaumburg, IL) is the only toxicant (nithiazine) baited with (Z)-9-tricosene that is registered for safe use around food preparation areas (Carlson & Hogsette, 2007). Additionally, physical control methods such as sticky tapes, which are commonly used in agriculture, are often unsuitable for use near food and in homes because the adhesive drips leave unwanted residue and the traps are visually displeasing to homeowners and restaurant guests (Carlson & Hogsette, 2007).

1.4.1. Sugar toxicants

House fly management in indoor agricultural (animal) facilities can involve sugar toxicant granules. These granules include an attractant such as (Z)-9-tricosene and an insecticide, often carbamates such as methomyl, or neonicotinoids such as imidacloprid and nithiazine (Butler *et al.,* 2007). Some common sugar toxicants are Golden Malrin™ (Wellmark International, Schaumburg, IL) which contains methomyl and QuickBayt™

(Bayer Healthcare, LLC, Shawnee, KS) which contains imidacloprid. Field studies of sugar toxicant baits show reduced efficacy of some longstanding insecticides, specifically methomyl baits, compared to newly-introduced ones (Butler *et al.,* 2007). The reduced efficacy is attributed to insecticide resistance development by house flies (Butler *et al.,* 2007). Evidence for this is described in more detail below.

1.4.2. Sticky traps

To avoid insecticides and the potential risk of resistance development by house flies, other large scale control methods are implemented. Large sticky cards and strips are utilized in indoor animal facilities to control and monitor house fly populations (Kaufman *et al.,* 2001, 2005). The strips are baited with (Z)-9-tricosene and left for days at a time to accumulate house flies. A major drawback of this tactic, specifically in dusty poultry houses, is that the strips are quickly covered with debris and thus become less effective at trapping house flies (Kaufman *et al.,* 2001). In certain situations, large-scale sticky traps can reduce house fly populations on farms (Kaufman *et al.,* 2001). However, sticky cards are more often used as a means of monitoring rather than controlling house fly populations (Goulson *et al.,* 1999; Kaufman *et al.,* 2005).

1.4.3. Baited trapping

Baited trapping is another common tactic for house fly control used in indoor animal rearing houses and in proximity to humans. In agriculture, large jug traps containing a drowning solution (water plus surfactant) are baited with an attractant and placed strategically around animal houses. House flies that enter through an opening in the trap eventually perish in the drowning solution. Many attractants serve as baits, ranging from food items to commercially available mixes (Geden, 2005). Jug traps are often left for several days before they need to be emptied and re-baited. Such traps can capture thousands of house flies at a time and can be used in IPM programs along with manure management, but trapping by itself should be considered a monitoring tool more than a control tactic in agriculture (Axtell & Arends, 1990; Geden, 2005).

Supplementary to refuse management, trapping is likely the most adequate method of house fly control for homes and restaurants. Once house flies are killed, they are out of sight and can be discarded easily. Attractants for traps often focus on ovipositional cues such as manure and rotting organic matter. Components of these innately malodourous substances have been identified and are now used in baited traps (Mulla *et al.,* 1977; Cossé & Baker, 1996). For instance, one attractant for a common jug trap, the Fly Terminator ® Trap, consists of (Z)-9-tricosene, trimethylamine, and indole (Geden *et al.,* 2009). Aside from the mostly odourless (Z)-9-tricosene, trimethylamine and indole are compounds identified from rotting meat and animal feces (Mulla *et al.,* 1977; Brodie *et al.,* 2016). These compounds are offensive to the human nose and would not be usable in a kitchen or around homes.

Food items have long been used as fragrant indoor house fly attractants. An incomplete list of such items includes mixtures of water and molasses, yeast, bread, vinegar, grain, milk, banana, apple, mango, honey, maple syrup, and vinegar (Pickens *et al.,* 1994; Smallegange, 2003; Quinn *et al.,* 2007; Albarrak, 2009; Geden *et al.,* 2009; Qian *et al.,* 2013). These products are commonly used but have rarely been analysed for their attractive components or put through efficacy trials. For instance, molasses is used in numerous studies as a positive control but the semiochemicals that attract house flies are not known (Carlson & Hogsette, 2007; Quinn *et al.,* 2007; Geden *et al.,* 2009). Floral resources also deserve to be investigated as potential house fly baits. Myophilous flies are important pollinators of many flowers (Larson *et al.,* 2001; Dobson, 2006) and likely respond to distinct floral semiochemicals when they seek floral nectar (Brodie *et al.,* 2015). Such compounds would likely lend themselves for deployment as indoor house fly baits.

The “fly factor” is yet another potential foraging cue that may be co-opted for use in fly management. The fly factor refers to the phenomenon that food currently or previously fed on by flies attracts more foraging flies than the same type and amount of food kept inaccessible to flies (Barnhart & Chadwick, 1953). The feeding activity by flies apparently enhances the recruitment and aggregation of conspecifics to a food patch (Danchin & Wagner, 1997; Lihoreau & Rivault, 2011). It is hypothesized that feeding flies produce and deposit regurgitate or feces that attracts foraging flies but no definitive

attractant has been identified in these sources despite various attempts (Barnhart & Chadwick, 1953; Dethier, 1955; Acree, 1959). Because the fly factor is imperceptible to the human nose, it may be perfectly suited as a trap bait for indoor attraction and capture of flies.

1.5. Resistance to common house fly baits

Chemical resistance to contact insecticides has been recorded in house flies as early as the 1940's when DDT (dichlorodiphenyltrichloroethane) resistance was first observed (Wiesmann, 1947 in Harrison, 1950). Since then, house flies have exhibited varying levels of resistance to most insecticides used against them (Mann *et al.,* 2010). Permethrin, a pyrethroid, is sprayed on animal shelters where adult house flies rest and contact the insecticide and has frequently been used for decades. Similar to DDT, the regular and arguably overuse of permethrin has resulted in significant levels of resistance around the world (Scott *et al.,* 2000; Kristensen *et al.,* 2001; Acevedo *et al.,* 2009).

1.5.1. Behavioural resistance to sugar toxicants

Sugar toxicant baits are commonly used on animal farms as an alternative to sprays. Toxicant-coated sugar granules are easier to deploy than sprays and can be kept in discrete bait stations away from people and animals for weeks at a time. Not surprisingly, resistance to baited toxicants has developed in adult house flies, with evidence suggesting that the resistance may be behavioural as well as physiological (Darbro & Mullens, 2004). Two-choice bioassays designed to give house flies the option of feeding on a sugar toxicant or on a non-toxic sugar alternative have shown that behavioural resistant populations avoid various toxic baits while non-resistant control populations will readily feed upon them (Darbro & Mullens, 2004; Gerry & Zhang, 2009).

House fly populations have exhibited resistance to some of the most commonly used toxic baits. Methomyl, a carbamate, has been used since the 1970's in toxic baits. Over time, the efficacy of methomyl baits has decreased. Laboratory experiments attribute this to both behavioural and physiological resistance (Learmount *et al.,* 2002;

Darbro & Mullens, 2004). More recently, alternatives to methomyl have been used, including neonicotinoids such as imidacloprid and nithiazine, and have been shown to induce resistance in house flies (Kaufman *et al.,* 2006; Mann *et al.,* 2010). Within just two years of registration and use, physiological resistance to imidacloprid developed (Kaufman *et al.,* 2006) quickly followed by behavioural resistance (Gerry & Zhang, 2009). Similarly, nithiazine, the only toxicant registered for use near food preparation, is losing its effectiveness to kill house flies (Carlson & Hogsette, 2007; Mann *et al.,* 2010).

As house flies are able to adapt to insecticides both through physiological and behavioural resistance, it may be prudent to shift the focus from insecticides to alternative control methods, especially because of the limited applicability of insecticides near human habitation and food. Baited trapping is a potential means to control house flies without insecticides and the risks associated with them.

1.6. Research Objective

Regulations for indoor house fly control tactics are stricter than those for outdoor tactics. The indoor tactic must consider a non-toxicant kill method, keep captured house flies out of view, and deploy attractants not offensive to the human nose.

The overall goal of my thesis is to investigate attractive (but ideally pleasantly smelling) foraging cues that house flies exploit to locate resources and that therefore could become effective indoor baits for fly traps. My specific goals were:

1. To investigate aromatic food products and floral resources that could potentially attract house flies in indoor settings (Chapter 2); and
2. To investigate the “fly factor” phenomenon in house flies (Chapter 3).

1.7. References

Acevedo, G.R., Zapater, M. and Toloza, A.C. (2009) Insecticide resistance of house fly, *Musca domestica* (L.) from Argentina. *Parasitology Research,* 105, 489-493.

Acree, F., Davis, P.L., Spear, S.F., LaBrecque, G.C. and Wilson, H.G. (1959) Nature of the attractant in sucrose fed on by house flies. *Journal of Economic Entomology,* 52, 981-985.

Adams, T.S. and Holt, G.G. (1987) Effect of pheromone components when applied to different models on male sexual behaviour in the house fly, *Musca domestica*. *Journal of Insect Physiology,* 33, 9-18.

Albarak, A.S. (2009) Comparative studies on house fly, *Musca domestica*, populations in different animal farms in relation to attractants and control at Hail Province, Saudi Arabia. *Pakistan Entomologist*, 31, 142-148.

Axtell, R.C. and Arends, J.J. (1990) Ecology and management of arthropod pests of poultry. *Annual Review of Entomology,* 35, 101-126.

Bahrndorff, S., Rangstrup-Christensen, L., Nordensoft, S. and Hald, B. (2013) Foodborne disease prevention and broiler chickens with reduced *Campylobacter* infection. *Emerging diseases,* 19, 425-430.

Barnhart, C.S. and Chadwick, L.E. (1953) A “fly factor” in attractant studies. *Science,* 117, 104-105.

Blaak, H., van Hoek, A.H.A.M., Hamidaja, R.A., van der Plaats, R.Q.J., Kerkhof-de Heer, L., de Roda Husman, A.M. and Schets, F.M. (2015) Distribution, numbers, and diversity of ESBL-producing *E. coli* in the poultry farm environment. *PLoS ONE,* 10: e0135402. doi:10.1371/journal.pone.0135402.

Brodie, B.S., Babcock, T., Gries, R. and Gries, G. (2016) Acquired smell? Mature females of the common green bottle fly shift semiochemical preferences from feces feeding sites to carrion oviposition sites. *Journal of Chemical Ecology,* 42, 40–50.

Brodie, B.S., Smith, M.A., Lawrence, J. and Gries, G. (2015) Effects of floral scent, color and pollen on foraging decisions and oocyte development of common green bottle flies. *PLoS ONE*, 10:e0145055. doi:10.1371/journal.pone.0145055.

Butler, J.F., Garcia-Maruniak, A., Meek, F. and Maruniak, J.E. (2010) Wild Florida house flies (*Musca domestica*) as carriers of pathogenic bacteria. *Florida Entomologist,* 93, 218-223.

Butler, S.M., Gerry, A.C. and Mullens, B.A. (2007) House fly (Diptera: Muscidae) activity near baits containing (Z)-9-tricosene and efficacy of commercial toxic fly baits on a southern California dairy. *Journal of Economic Entomology,* 100, 1489-1495.

Butler, S.M. and Mullens, B.A. (2010) Adult house fly (Diptera: Muscidae) activity and age of females near varying levels of (Z)-9-tricosene on a southern California dairy. *Journal of Economic Entomology,* 103, 1929-1936.

Carlson, D.A. and Beroza, M. (1973) Field evaluation of (Z)-9-tricosene, a sex attractant pheromone of the house fly. *Environmental Entomology,* 2, 555-559.

Carlson, D.A. and Hogsette, J.A. (2007) Flybrella: A device to attract and kill house flies. *Journal of Economic Entomology,* 100, 483-487.

Carlson, D.A. and Leibold, C.M. (1981) Field trials of pheromone-toxicant device containing muscalure for house flies (Diptera: Muscidae). *Journal of Medical Entomology,* 18, 73-77.

Carlson, D.A., Mayer, M.S., Silhacek, D.L., James, J.D., Beroza, M., Bierl, B.A. and Calam, D.H. (1971) Sex attractant pheromone of the house fly: Isolation, identification and synthesis. *Science,* 174, 76-78.

Chapman, J.W., Howse, P.E., Knapp, J.J. and Goulson, D. (1998a) Evaluation of three (Z)-9-tricosene formulations for control of *Musca domestica* (Diptera: Muscidae) in caged-layer poultry units. *Journal of Economic Entomology,* 91, 915-922.

Chapman, J.W., Knapp, J.J., Howse, P.E. and Goulson, D. (1998b) An evaluation of (Z)-9-tricosene and food odours for attracting *Musca domestica,* to baited targets in deep-pit poultry units. *Entomologia Experimentalis et Applicata,* 89, 183-192.

Chapman, J.W., Knapp, J.J. and Goulson, D. (1999) Visual responses of *Musca domestica* to pheromone impregnated targets in poultry units. *Medical and Veterinary Entomology,* 13, 132-138.

Christensen, B., Sommer, H., Rosenquist, H. and Neilsen, N. (2001) Risk assessment on *Campylobacter jejuni* in chicken products. The Danish Veterinary and Food Administration Institute of Food Safety and Toxicology Division of Microbial Safety. 1st Edition.

Cirillo, V.J. (2006) “Winged Sponges”: House flies as carriers of typhoid fever in 19th- and early 20th-century military camps. *Perspectives in Biology and Medicine,* 49, 52-63.

Cohen, D., Green, M., Block, C., Slepon, R., Ambar, R., Wasserman, S.S. and Levine, M. (1991) Reduction of transmission of shigellosis by control of house flies (*Musca domestica*). *The Lancet,* 337, 993-997.

Danchin, E. and Wagner, R.H. (1997) The evolution of coloniality: the emergence of new perspectives. *Trends in Ecology & Evolution*, 12, 342–347.

Darbro, J.M. and Mullens, B.A. (2004) Assessing insecticide resistance and aversion to methomyl-treated toxic baits in *Musca domestica* L (Diptera: Muscidae) populations in southern California. *Pest Management Science,* 60, 901-908.

Darbro, J.M., Millar, J.G., McElfresh, J.S. and Mullens, B.A. (2005) Survey of muscalure [(Z)-9-tricosene] on house flies (Diptera: Muscidae) from field populations in California. *Environmental Entomology,* 34, 1418-1425.

De Jesús, A., Olsen, A.R., Bryce, J.R. and Whiting, R.C. (2004) Quantitative contamination and transfer of *Escheria coli* from foods by house flies, *Musca domestica* L. (Diptera: Muscidae). *International Journal of Food and Microbiology*, 93, 259-262.

Dethier, V.G. (1955) Mode of action of sugar-baited fly traps. *Journal of Economic Entomology,* 48, 235-239.

Dobson, H.E.M. (2006) Relationship between floral fragrance composition and type of pollinator in *Biology of floral scent*. Ed. Dudareva, N. and Pichersky, E. CRC Press, Boca Raton, FL, Pp. 147-198.

Fasanella, A., Scasciamacchia, S., Garofolo, G., Giangaspero, A., Tarsitano, E. and Adone, R. (2010) Evaluation of the house fly *Musca domestica* as a mechanical vector for an anthrax. *PLoS ONE,* 5: e12219. doi:10.1371/journal.pone.0012219.

Förster, M., Klimpel, S., Mehlhorn, H., Sievert, K., Messler, S. and Pfeffer, K. (2007) Pilot study on synanthropic flies (e.g. *Musca*, *Sarcophaga, Calliphora, Fannia, Lucilia, Stomoxys*) as vectors of pathogenic microorganisms. *Parasitology Research*, 101, 243-246.

Geden, C.J. (2005) Methods for monitoring outdoor populations of house flies, *Musca domestica* L. (Diptera: Muscidae). *Journal of Vector Ecology,* 30, 244-250.

Geden, C.J., Szumlas, D.E. and Walker, T.W. (2009) Evaluation of commercial and field-expedient baited traps for house flies, *Musca domestica*, L. (Diptera: Muscidae). *Journal of Vector Ecology*, 34: 99-103.

Gerry, A.C. and Zhang, D. (2009) Behavioral resistance of house flies, *Musca domestica* (Diptera: Muscidae) to imidacloprid. *The United States Medical Department Journal - Operational and Public Health Entomology*, July-September 2009, 54-59

Gill, C. (2014) Investigating the vector competence of the house fly (*Musca domestica*) for *Campylobacter jejuni.* Master of Science Thesis, Simon Fraser University, Burnaby, British Columbia, Canada, pp. 83-115.

Gill, C., Bahrndorff, S. and Lowenberger, C. (2016) *Campylobacter jejuni* in *Musca domestica*: An examination of survival and transmission potential in light of the innate immune response of the house flies. *Insect Science*, doi: 10.1111/1744-7917.12353.

Goulson, D., Hughes, W.H.O. and Chapman, J.W. (1999) Fly populations associated with landfill and composting sites used for household refuse disposal. *Bulletin of Entomological Research,* 89, 493-498.

Greenberg, B. (1964) Experimental transmission of *Salmonella typhimurium* by house flies to man. *The American Journal of Hygiene*, 80, 149-156.

Greenberg, B. (1971) Flies and Disease Vol. 1: Ecology, classification and biotic associations. Princeton: Princeton University Press. pp. 17-54.

Gupta, A.K., Nayduch, D., Verma, P., Shah, B., Ghate, H.V., Patole, M.S. and Schouce, Y.S. (2011) Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica* L.). *FEMS Microbial Ecology*, 79, 581–593.

Hald, B., Skovgård, H., Bang, D.D., Pedersen, K., Dybdahl, J., Jespersen, J.B. and Madsen, M. (2004) Flies and *Campylobacter* infection of broiler flocks. *Emerging Infectious Disease,* 10, 1490-1492.

Hald, B., Skovgård, H., Pedersen, K. and Bunkenborg, H. (2008) Influxed insects as vectors for *Campylobacter jejuni* and *Campylobacter coli* in Danish broiler houses. *Poultry Science,* 87, 1428-1434.

Hanley, M.E., Cruickshanks, K.L., Dunn, D., Stewart-Jones, A. and Goulson, D. (2009) Luring house flies (*Musca domestica*) to traps: Do cuticular hydrocarbons and visual cues increase catch? *Medical and Veterinary Entomology,* 23, 26-33.

Hanley, M.E., Dunn, D.W., Abolins, S.R. and Goulson, D. (2004) Evaluation of (Z)-9-tricosene baited targets for control of the house fly (*Musca domestica)* in outdoor situations. *Journal of Applied Entomology,* 128, 478-482.

Harrison, C.M. (1950) DDT-resistant house flies. *Nature,* 37, 306-309.

Kaufman, P.E., Gerry, A.C., Rutz, D.A. and Scott, J.G. (2006) Monitoring susceptibility of house flies (*Musca domestica* L.) in the United States to imidacloprid. *Journal of Agricultural and Urban Entomology*, 23, 195-200.

Kaufman, P.E., Rutz, D.A. and Frish, S. (2001) Sticky traps for large scale house fly (Diptera: Muscidae) trapping in New York poultry facilities. *Journal of Agricultural and Urban Entomology,* 18, 43-49.

Kaufman, P.E., Rutz, D.A. and Frish, S. (2005) Large sticky traps for capturing house flies and stable flies in dairy calf greenhouse facilities. *Journal of Dairy Science,* 88, 176-181.

Kristensen, M., Spencer, A.G. and Jespersen, J.B. (2001) The status and development of insecticide resistance in Danish populations of the house fly *Musca domestica* L. *Pest Management Science,* 57, 82-89.

Larson, B.H.M., Kevan, P.G. and Inouye, D.W. (2001) Flies and flowers: Taxonomic diversity of anthophiles and pollinators. *The Canadian Entomologist,* 133, 439-465.

Learmount, J., Chapman, P. and MacNicoll, A. (2002) Impact of insect resistance strategy for house fly (Diptera: Muscidae) control in intensive animal units in the United Kingdom. *Journal of Economic Entomology,* 95, 125-1250.

Levine, O.S. and Levine, M.M. (1991) House flies (*Musca domestica*) as mechanical vectors of shigellosis. *Reviews of Infectious Diseases,* 13, 688-696.

Lihoreau, M. and Rivault, C. (2011) Local enhancement promotes cockroach feeding aggregations. *PLoS ONE*, 6, 1–7.

Mann, R.S., Kaufman, P.E. and Butler, J.F. (2010) Evaluation of semiochemical toxicity to house flies and stable flies (Diptera: Muscidae). *Pest Management Science,* 66, 816-824.

Meerburg, B.G., Vermeer, H.M. and Kijlstra, A. (2007) Controlling risks of pathogen transmission by flies on organic pig farms – A review. *Outlook on Agriculture,* 36, 193-197.

Mian, L.S., Maag, H. and Tacal, J.V. (2002) Isolation of *Salmonella* from muscoid flies at commercial animal establishments in San Bernardino County, California. *Journal of Vector Ecology,* 27, 82-85.

Moon, R.D. and Meyer, H.J. (1985) Nonbiting flies, in *Livestock Entomology*, ed by Williams RE, Hall RD, Brose AB & School PJ. John Wiley and Sons, New York, USA, pp 65-82.

Morgan, P.B., Gilbert, I.H. and Fye, R.L. (1974) Evaluation of (Z)-9-tricosene for attractancy for *Musca domestica* in the field. *The Florida Entomologist,* 57, 136-140.

Moriya, K., Fujibayashi, T., Yoshihara, T., Matsuda, A., Sumi, N., Umezaki, N., Kurahasi, H., Agui, N. and Wada, A. (1999) Verotoxin-producing *Escherichia coli* O157:H7 carried by the house fly in Japan. *Medical and Veterinary Entomology,* 13, 214-216.

Mulla, M.S., Hwang, Y.S. and Axelrod, H. (1977) Attractants for synanthropic flies: Chemical attractants for domestic flies. *Journal of Economic Entomology,* 70, 644-648.

Mullen, G.R., Durden, L.A. (2009) Muscid flies: Species of medical-veterinary importance in *Medical and Veterinary Entomology, 2nd Edition.* Academic Press. p. 288.

Nanzi, W.A., Seleena, B., Lee, H.L., Jeffery, J., T. Rogayah, T.A.R. and Sofian, M.A. (2005) Bacteria fauna from the house fly, *Musca domestica (L.)*. *Tropical Biomedicine*, 22, 225-231.

Nichols, G.L. (2005) Fly transmission of *Campylobacter*. *Emerging Infectious Disease,* 11, 361-364.

Noorman, N. and den Otter, C.J. (2001) The effects of laboratory culturing on (Z)-9-tricosene (muscalure) quantities on female house flies*. Entomologia Experimentalis et Applicata,* 101, 69-80.

Olsen, A.R. (1998) Regulatory action criteria for filth and other extraneous materials: III. Review of flies and foodborne enteric disease. *Regulatory Toxicology and Pharmacology,* 28, 199-211.

Olsen, A.R., Gecan, J.S., Ziobro, G.C. and Bryce, J.R. (2001) Regulatory action criteria for filth and other extraneous materials: V. Strategy for evaluating hazardous and nonhazardous filth. *Regulatory Toxicology and Pharmacology,* 33, 363-392.

Pedigo, L.P. and Rice, M.E. (2009) Entomology and pest management, 6th ed. Pearson Education, Inc., Columbus, Ohio, USA. pp 530.

Pickens, L.G., Schmidtmann, E.T. and Miller, R.W. (1994) How to control house and stable flies without using pesticides. US Department of Agriculture, Agriculture Information Bulletin No. 673, p. 14.

Qian, K., Zhu, J.J., Sims, S.R., Taylor, D.B. and Zeng, X. (2013) Identification of volatile compounds from a food-grade vinegar attractive to house flies (Diptera: Muscidae). *Journal of Economic Entomology*, 106, 979-987.

Quinn, B.P., Bernier, U.R., Geden, C.J., Higsette, J.A. and Carlson, D.A. (2007) Analysis of extracted and volatile components in blackstrap molasses feed as candidate house flu attractants. *Journal of Chromatography A*, 1139, 279-284.

Rogoff, W.M., Beltz, A.D., Johnsen, J.O. and Plapp, F.W. (1964) A sex pheromone in the house fly, *Musca domestica* L. *Journal of Insect Physiology,* 10, 239-246.

Rogoff, W.M., Gretz, G.H., Jacobson, M. and Beroza, M. (1973) Confirmation of (Z)-9-tricosene as a sex pheromone of the house fly. *Annals of the Entomological Society of America*, 66, 739-741.

Rogoff, W.M., Gretz, G.H., Sonnet, P.F. and Schwarz, M. (1980) Response of male house flies to muscalure and to combinations of hydrocarbons with and without muscalure. *Environmental Entomology,* 9, 605-606.

Rosef, O. and Kapperud, G. (1983) House flies (*Musca domestica*) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. *Applied and Environmental Microbiology,* 45, 381-383.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L. and Griffin, P.M. (2011) Foodborne illness acquired in the United States – Major pathogens. *Emerging Infectious Disease,* 17, 7-15.

Scott, J.G., Alefantis, T.G., Kaufman, P.E. and Rutz, D.A. (2000) Insecticide resistance in house flies from caged-layer poultry facilities. *Pest Management Science,* 56, 147-153.

Shane, S.M., Montrose, M.S. and Harrington, K.S. (1984) Transmission of *Campylobacter jejuni* by the house fly (*Musca domestica*). *Avian Disease,* 29, 383-391.

Smallegange, R.C. (2003) Attractiveness of different light wavelengths, flicker frequencies and odours to the house fly (*Musca domestica* L.). Ph.D. Thesis. University of Groningen, The Netherlands.

Szalanski, A.L., Owens, C.B., McKay, T. and Steelman, C.D. (2004) Detection of *Campylobacter* and *Escherichia coli* O157:H7 from filth flies by polymerase chain reaction. *Medical and Veterinary Entomology,* 18, 241-246.

Uebel, E.C., Sonnet, P.E. and Miller, R.W. (1976) House fly sex pheromone: Enhancement of mating strike activity by combination of (Z)-9-tricosene with branched saturated hydrocarbons. *Environmental Entomology,* 5, 905-908.

Ugbogu, O.C., Nwachukwu, N.C. and Ogbuagu, U.N. (2006) Isolation of *Salmonella* and *Shigella* species from house flies (*Musca domestica* L.) in Uturu, Nigeria. *African Journal of Biotechnology*, 5, 1090-1091.

Wiesmann, V.R. (1947) Untersuchungen über das physiologishe Verhalten von *Musca domestica* L. vershiedener Provenienzen. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft,* 20, 484.

Wang, Y.C., Chnag, Y.C., Chuang, H.L., Chiu, C.C., Yeh, K.S., Chang, C.C., Hsuan, S.L., Lin, W.H. and Chen, T.H. (2011) Trasmission of *Salmonella* between swine farms by the house fly (*Musca domestica*). *Journal of Food Protection,* 74. 1012-1016.

Chapter 2.

Investigating human food items and flowers as attractants for house flies (*Musca domestica*)

2.1. Abstract

Control programs for pesticide-resistant house flies (*Musca domestica* L.) have integrated attract-and-kill tactics. Although fecal and cadaverous odorants attract house flies, they could not be used as fly lure in indoor settings. My research objective was to investigate aromatic food and floral resources that might be deployable for indoor attraction of house flies. In still-air, two-choice laboratory bioassays, I tested the responses of house flies to blackstrap molasses, pale ale beer, Honeycrisp apples, apple cider vinegar, dandelion honey, and dandelion flowers (*Taraxacum officinale* L.). Of all these, only dandelion flowers and dandelion honey attracted house flies. All analytical attempts to capture the essential semiochemicals of dandelion flowers and honey failed, highlighting the need to develop alternative analytical approaches.

Key words *Musca domestica*, trapping, indoor, semiochemical, foraging, dandelion, honey

2.2. Introduction

Pesticide-resistant house flies (*Musca domestica* L.) prompted a shift in fly control programs from pesticides to attract-and-kill tactics (Hanley *et al.,* 2009). Attract-and-kill fly control tactics require effective attractants which often originate from essential foraging or oviposition resources of flies [human food, rotting organic matter, silage, and manure (Mulla *et al.,* 1977; Pickens *et al.,* 1994; Cossé & Baker, 1996; Smallegange, 2003; Geden *et al.,* 2009)] or pheromonal communication signals of flies [(Z)-9-tricosene

(Carlson *et al.,* 1971; Adams & Holt, 1986; Butler & Mullens, 2010)]. Despite its inconsistent attractiveness, (Z)-9-tricosene (commercially known as muscalure or muscamone) is used as an attractant in house fly traps and in attract-and-kill compositions (Chapman *et al.,* 1998; Butler *et al.,* 2007; Hanley *et al.,* 2004, 2009).

Many malodourous food and oviposition resources of flies emanate semiochemicals attractive to flies. Putrefying meat, for example, emits indole and trimethylamine which are each attractive to house flies when tested against a blank control (Mulla *et al.,* 1977) but a blend of indole, trimethylamine, linoleic acid, and ammonia is even more attractive (Mulla *et al.,* 1977). A 3-component blend of butanoic acid, skatole (3-methylindole), and dimethyltrisulfide identified in pig manure attracted nearly as many female house flies as did pig manure (Cossé & Baker, 1996).

Even though malodourous semiochemicals attract house flies, they are not suitable for deployment in homes. For example, butanoic acid is considered "contraindicated due to its bad odor" (Mulla *et al.,* 1977) and indole has a musty fecal, mothball, or burnt smell (Laor *et al.,* 2015). Finding semiochemicals that adequately attract house flies while not being off-putting to the human nose is a major challenge.

Human food products have long been used as fragrant indoor fly baits. Such products include, but are not limited to, mixtures of water and molasses, yeast, bread, vinegar, grain, milk, banana, apple, mango, honey, maple syrup and vinegar (Pickens *et al.,* 1994; Smallegange, 2003; Quinn *et al.,* 2007; Albarrak, 2009; Geden *et al.,* 2009; Qian *et al.,* 2013). Although commonly used for house fly control, these products are rarely tested for their effect on attraction of flies or rarely analysed for the semiochemicals that attract the flies. For instance, molasses is used in numerous studies as a positive control but the semiochemicals that attract house flies are not known (Carlson & Hogsette, 2007; Quinn *et al.,* 2007; Geden *et al.,* 2009). A synthetic semiochemical lure would avoid complications associated with food baits such as spoilage and relatively high costs.

Floral resources also warrant investigations for fly semiochemicals. Dipterans are part of the pollination regime in many myophilous flowers (Larson *et al.,* 2001; Dobson, 2006; Brodie *et al.,* 2015). Myophilous inflorescences attract fly pollinators with sweet

odours indicative of nectar rewards, often displaying an open bowl-shaped morphology that facilitates nectar and pollen feeding by short-tongued (house) flies (Dafni, 1984; Dobson, 2006). The common dandelion (*Taraxacum officinale* L.) meets the physical criteria of a myophilous flower. It has an open flower face, abundant pollen and nectar to reward pollinators, and it is frequented by many flies (Muñoz & Cavieres, 2008). For these reasons, dandelion flowers were investigated for attractiveness to house flies.

My objectives in this chapter were to bioassay aromatic food products with a yeasty, fermenting, or sweet scent (blackstrap molasses, dandelion honey, pale ale beer, Honeycrisp apples, apple cider vinegar) deemed attractive to house flies (Hwang *et al.,* 1978; Smallegange, 2003; Quinn *et al.,* 2007; Albarak, 2009; Geden *et al.,* 2009; Qian *et al.,* 2013) as well as a floral resource (the common dandelion) for attraction of house flies, and determine whether essential semiochemicals of proven attractive resources can be captured in solvent or headspace volatile extracts of these resources for attraction of house flies.

2.3. Materials and methods

2.3.1. Experimental insects

The adult house flies that were tested in experiments over the course of three years originated either from a laboratory-reared strain purchased from a supplier (Beneficial Insectary Inc., Redding, CA, USA) or from maggots collected at a local chicken production facility in Abbotsford, BC, Canada. Flies were reared in the insectary at Simon Fraser University (Burnaby, BC, Canada) at 25 °C, 60% RH, and a photoperiod of L16:D8. Adult flies were provisioned with milk powder, table sugar, and water *ad libitum*. Larvae were reared in 4-L glass jars containing a mixture of wheat bran (2 L), molasses (40 mL), water (700 mL), dry brewer's yeast (20 mL), and thick milk powder paste (50 mL). Bioassays were run with 3- to 5-day-old adult flies that were starved for 16 h prior to bioassays. Although purchased and wild-type flies responded similarly to identical test stimuli, we ran most experiments with wild type flies.

2.3.2. General bioassay design

Two-choice laboratory bioassays were run in 61-cm^3 insect rearing cages (BioQuip Products, Compton, CA, USA) illuminated from above by fluorescent lights (Philips F32TA, Amsterdam, The Netherlands). On opposite sides of each cage were two inverted glass funnel bottle traps (Fig. 2.1A). The traps were made from 0.5-L glass bottles (7 cm i.d × 18 cm tall) that were cut 10 cm above the base, thus creating a 10-cm tall glass cylinder and an 8-cm tall funnel. The funnel was inverted and placed atop the cylinder to guide flies into the cylindrical base and discourage them from leaving. Each bottle trap was wrapped in yellow paper (color: canary, Hammermill International Paper Company, item #103341; Memphis, TN, USA) to occlude visual cues within traps. Experimental stimuli were contained inside the traps and were typically held in a metal mesh-covered glass vial (20 mL) wire-suspended 2 cm above unscented soapy water for drowning responding flies (Fig. 2.1A). For each replicate, flies were briefly (5 min) cold-sedated and sorted into mixed-sex groups of 50 flies which were then given 10 min to warm to room temperature and subsequently released into the cage. These flies, termed here “response flies” (RFs), were given a choice between the two bottle traps each containing a randomly assigned test stimulus. After 3 h, the traps were removed from the cages and captured RFs counted.

2.3.3. Objective 1: Determine the attractiveness of food products and a floral resource to house flies

Experiments 1-6 (Table 2.1) were designed to test a range of food products and floral resources for attraction of house flies. Experiment 1 (N = 20) tested diluted dandelion honey (Wedderspoon® 100% Raw Organic Wild Dandelion Honey, Duncan, BC, Canada; Product of NZ) [10 mL of diluted honey; honey (2): distilled water (1)] *versus* a distilled water control (10 mL). Experiment 2 (N = 20) tested dandelion flowers collected on the Burnaby campus of Simon Fraser University (SFU). Two cut flowers were placed in a 20-mL vial filled with distilled water which was then suspended within the inverted funnel trap and tested *versus* an empty-vial control. Experiment 3 (N = 20) tested 5 mL of pure apple cider vinegar (Heinz® Apple Cider Vinegar, H.J. Heinz Company, L.P., Pittsburgh, PA, USA) *versus* an empty-vial control. Experiment 4 (N = 10) tested 5 mL of diluted blackstrap molasses (Crosby’s Molasses

Co. Ltd., Saint John, NB, Canada) [molasses (1): distilled water (1)] *versus* a 5-mL distilled water control. Experiment 5 (N = 10) tested 5 mL of flat (air-exposed for 24 h) pale ale beer (Okanagan Spring Brewery Pale Ale, Vernon, BC, Canada) *versus* an empty-vial control. Experiment 6 (N = 10) tested slices of Honeycrisp apples (3 g, skinless) *versus* an empty trap.

2.3.4. Objective 2: Determine whether essential semiochemicals of proven effective sources can be captured in solvent or headspace volatile extracts for attraction of house flies

With evidence that dandelion honey attracts house flies (Exp. 1, see Results), dandelion headspace volatiles were captured. To this end, diluted honey (280 g of honey in 100 mL of distilled water) was poured into a horizontal glass chamber (10 cm i.d × 27 cm long; Fig. 2.1B) and charcoal-filtered air was drawn at 0.4 L/min for 24 h through the chamber and a glass tubing (0.5 cm i.d. × 12 cm long) containing 6 g of Porapak-Q (50-80 mesh, Waters Associates Inc. Milford MA). Volatiles were desorbed (extracted) from the Porapak-Q with 2 mL of pentane. This process was repeated three times, resulting in 20,160 Gram-Hour-Equivalents-of-Honey (GHEHs) of volatile release (24 GHEHs = the amount of volatiles emitted from 1 g of diluted honey during a 24-h period). Extracts were passively concentrated to 2.4 GHEH/µL.

Experiment 7 (N = 22) tested the attractiveness of headspace volatile extract of honey *versus* a pentane control. Aliquots (58 µL) of extract equivalent to 196 GHEHs were pipetted into a 20-mL vial suspended within the trap and allowed to evaporate completely before starting the bioassay. A pentane control was processed analogously.

With evidence that headspace volatile extracts of diluted dandelion honey were not attractive to flies (Exp. 7, see Results), dandelion honey was extracted using continuous liquid-liquid extraction (Pavia *et al.,* 1999a) with dichloromethane (DCM). To that end, honey (56 g) was mixed with distilled water (60 mL) and extracted with DCM. The DCM extract was collected and concentrated to 5.6 Gram-Extract-Equivalents-of-Honey (GEEHs; 1 GEEH = the amount of materials including odorants extracted from 1 g of honey present in 100 µl of DCM). The process was done four times total and batches were combined.

Experiment 8 (N = 20) tested the attractiveness of DCM honey extract *versus* a DCM control. Aliquots (200 µL) of DCM honey extract, equivalent to 11.2 GEEHs, were pipetted into a 20-mL vial suspended within the trap and allowed to evaporate completely before starting the bioassay. The DCM control (200 µL) was processed analogously.

With evidence that the DCM dandelion honey extract did not attract flies (Exp. 8, see Results), diluted honey was extracted using simple distillation (Pavia *et al.,* 1999b) as yet another means of extracting volatiles. Dandelion honey (84 g) was mixed with distilled water (30 mL) and poured into a 2-L round-bottomed Pyrex® flask (166 mm diam.) boiling the mixture for 4 h. The distillate was collected at a concentration of 1.68 Gram-Distillate-Equivalents-of-Honey (GDEHs; 1 GDEH = the amount of materials including odorants distilled from 1 g of honey present in 100 µl of distillate). The process was repeated once and batches were combined.

Experiment 9 (N = 10) tested the dandelion honey distillate for attraction of house flies. Aliquots (1 mL), equivalent to 16.8 GDEHs, were bioassayed *versus* a distilled-water control (1 mL) following the “general bioassay design”. The water-based distillate did not significantly evaporate prior to or during bioassays.

With evidence that dandelion flowers attract house flies (Exp. 2, see Results), headspace volatiles of dandelion flowers were captured. Dandelion flowers were collected on SFU’s Burnaby campus. Bundles of about 100 cut dandelion flowers were placed into a glass jar (140 mm high × 75 mm i.d.) filled with distilled water which was then inserted into a Pyrex® glass chamber (340 mm high × 125 mm wide) (Fig. 2.1C). Charcoal-filtered air was drawn at 0.4 L/min for 24 h through the chamber and a glass tubing (5 cm i.d. × 120 mm) containing 6 g of Porapak-Q. Volatiles were desorbed from the Porapak-Q with 1 mL of pentane. The process was repeated 10 times, creating a batch equal to 24,000 Flower-Hour-Equivalents (FHEs) of volatile emission (1 FHE = the amount of volatiles emitted from 1 flower in 1 h). Extracts were passively concentrated to 24 FHEs/µL. Two aerations batches were produced, one in spring of 2013 and one in spring of 2014.

Experiments 10-12 tested the attractiveness of various concentrations of headspace volatile extract of dandelion flowers. Following the “general bioassay design”, aliquots of 24 FHEs (Exp. 10, N = 16), 240 FHEs (Exp. 11, N = 20), and 2,400 FHEs (Exp. 12, N = 10) were each tested in two-choice tests *versus* a pentane control. For each test, aliquots [Exp. 10: 10 µL of diluted extract (extract (1): pentane (9)); Exp. 11: 10 µL; Exp. 12: 100 µL)] were pipetted into 20-mL vials suspended within traps and allowed to evaporate completely before starting the bioassay. Pentane controls were processed accordingly.

With evidence that headspace volatile extracts of dandelion flowers are not attractive to flies (Exps. 10-12, see Results), the inflorescences themselves were solvent-extracted. Ten freshly cut inflorescences were placed in a 250-mL beaker filled with 125 mL of DCM such that they were completely submerged. The stems were forced through a small slit in a Parafilm M® (Bemis Company, Inc., Oshkosh, WI, USA) sheet stretched over the top of the beaker to slow DCM evaporation. After 22 h, the inflorescences were removed and the solvent extract was concentrated to 1 Flower-Extract-Equivalent (FEE; 1 FEE = the amount of materials including odorants extracted from 1 flower present in 100 µL of DCM).

Experiment 13 (N = 10) tested the DCM dandelion flower extract *versus* a DCM control. Aliquots (100 µL), equivalent to 1 FEE, were pipetted into a 20-mL vial suspended within the trap and allowed to evaporate completely prior to the beginning of bioassays. A DCM control (100 µL) was processed analogously.

With evidence that the DCM dandelion flower extract did not contain the volatiles attractive to flies (Exp. 13, see Results), dandelion flowers were distilled. Approximately 100 inflorescences were placed in a 2-L round-bottomed Pyrex® flask (166 mm diam.) and submerged in distilled water (150 mL), boiling the water for 4 h. The distillate was collected at a concentration of 0.4 Flower-Distillate-Equivalents (FDEs; 1 FDE = the amount of materials including odorants distilled from 1 flower in 100 µL of distillate). The process was done four times total and batches were combined.

Experiment 14 (N = 10) tested the dandelion flower distillate for attraction of house flies. Aliquots (2 mL), equivalent to 8 FDEs, were tested *versus* a distilled water

control (2 mL) following the “general bioassay design”. The water-based distillate did not significantly evaporate prior to or during the bioassays.

2.3.5. Statistical analyses

In each of two-choice experiments 1-14, the proportions of RFs that were captured in traps were analyzed as a complete randomized block design where each cage was considered a block. The mean proportion of RFs to a treatment stimulus was compared by one-sample t-test to an expected equal (0.5) proportion of RFs to each of the two test stimuli. All data were analyzed by JMP 12 (SAS Institute Inc.).

2.4. Results

2.4.1. Objective 1: Determine the attractiveness of food products and a floral resources to house flies

The proportion of RFs (mean ± SE) responding to the diluted dandelion honey (0.63 ± 0.03) was significantly higher than the proportion of RFs responding to the control ($t = 3.5049$, $P < 0.005$; Fig. 2.2, Exp. 1). The proportion of RFs responding to dandelion flowers (0.67 ± 0.05) was significantly higher than the proportion of RFs responding to the control ($t = 4.0551$, $P < 0.001$; Fig. 2.2, Exp. 2). The proportion of RFs responding to the apple cider vinegar (0.56 ± 0.05) did not significantly differ from the proportion of RFs responding to the control ($t = 1.2233$, $P = 0.2362$; Fig. 2.2, Exp. 3). The proportion of RFs responding to diluted molasses (0.31 ± 0.10) did not significantly differ from the proportion of RFs responding to the control ($t = -1.7321$, $P = 0.1173$; Fig. 2.2, Exp. 4). The proportion of RFs responding to the flat pale ale beer (0.48 ± 0.08) did not significantly differ from the proportion of RFs responding to the control ($t = -0.1991$, $P = 0.8500$; Fig. 2.2, Exp. 5). The proportion of RFs responding to Honeycrisp apples (0.43 ± 0.12) did not significantly differ from the proportion of RFs responding to the control ($t = -0.5241$, $P = 0.6128$; Fig. 2.2, Exp. 6).

2.4.2. Objective 2: Determine whether essential semiochemicals of proven effective sources can be captured in solvent or headspace volatile extracts for attraction of house flies

The proportion of RFs (mean ± SE) responding to the dandelion honey aeration (0.57 ± 0.05) was not different than the proportion of RFs responding to the control ($t = 1.3773$, $P = 0.1829$; Fig. 2.3, Exp. 7). The proportion of RFs responding to the liquid-liquid DCM extract of dandelion honey (0.49 ± 0.04) was not significantly different from the proportion of RFs responding to the DCM control ($t = -0.2407$, $P = 0.812$; Fig. 2.3, Exp. 8). The proportion of RFs responding to the distillate of dandelion honey (0.47 ± 0.09) was not different from the proportion of RFs responding to the control ($t = -0.3505$, $P = 0.7340$; Fig. 2.3, Exp. 9). The proportions of RFs responding to Porapak-Q headspace volatile extract of dandelion flowers (Fig. 2.4) at 24 FHEs (0.44 ± 0.07; Exp. 10), at 240 FHEs (0.33 ± 0.05; Exp. 11) or at 2,400 FHEs (0.33 ± 0.09; Exp. 12), were all not significantly higher than the proportions of RFs responding to the corresponding control (Exp. 10: $t = -0.8438$, $P = 0.4120$; Exp. 11: $t = -3.3398$, $P < 0.01$; Exp. 12: $t = -1.995$, $P = 0.0766$). In contrast, the proportion of RFs responding to the DCM extract of dandelion flowers (0.31 ± 0.05) was significantly lower than the proportion of RFs responding to the DCM control ($t = -3.9301$, $P < 0.005$; Fig. 2.4, Exp. 13). The proportion of RFs responding to the distillate of dandelion flowers (0.48 ± 0.06) was not different from the proportion of RFs responding to the water control ($t = -0.3746$, $P = 0.7166$; Fig. 2.4, Exp. 14).

2.5. Discussion

I present evidence that both dandelion flowers and dandelion honey attract house flies in laboratory experiments (Fig. 2.2, Exps. 1, 2). In contrast, Porapak-Q headspace volatile extract from both dandelion honey (Fig. 2.3, Exp. 7) and dandelion flowers (Fig. 2.4, Exps. 10-12) failed to attract house flies, as did other analytes including liquid-liquid extracts and distillates of dandelion honey (Fig. 2.3, Exps. 8, 9), and DCM extracts and distillates of dandelion flowers (Fig. 2.4, Exps. 13, 14).

The attractive semiochemicals of dandelion flowers and dandelion honey are possibly too volatile to be acquired by the techniques I applied in my study. Sapromyophilous flowers that attract flies for pollination sometimes produce fecal and cadaverous odorants of such low molecular weight that these odorants may be detectable only by cryogenic trapping or cold on-column gas chromatograph (GC) injection of extract aliquots (Raguso, 2004). Considered not to be sapromyophilic, dandelions have an unspecialized pollination syndrome and are visited by a range of insect taxa (Muñoz & Cavieres, 2008). The diverse volatiles dandelions disseminate (Piasenzotto *et al.,* 2003; Bylka *et al.,* 2010) are probably appealing to a wide range of potential pollinators. Sweet-smelling odorants might attract nectar-foraging myophilous insects, whereas fecal or cadaverous odorants may attract sapromyophilous gravid filth flies including house flies (Dobson, 2006). If so, these types of compounds would not likely be suitable as a lure for indoor fly traps.

Aside from honey, none of the other food items I tested were attractive to house flies (Fig. 2.2). Considering that house flies feed on a generalist diet, these results were not expected and contrast with previously reported findings that house flies are attracted to blackstrap molasses (Quinn *et al.,* 2007), vinegar (Qian *et al.,* 2013), and apples (Smallegange, 2003).

The efficacy of molasses may depend upon its specific grade. Food-grade molasses processed for human consumption had no effect on house fly attraction in my study (Fig. 2.1, Exp. 4) and totally failed as a bait in house fly traps (Geden, 2005). In contrast, animal-feed-grade molasses was as attractive as a commercial house fly lure (Geden, 2005) and attracted house flies in laboratory experiments (Quinn *et al.,* 2007). Because most studies do not specify the grade of molasses that was tested, it is difficult to determine the extent to which the grade factor is relevant but it seems to be important (Albarrak, 2009; Geden *et al.,* 2009).

The variety of vinegar also appears to affect its attractiveness to house flies. Qian *et al.* (2013) showed that rice vinegar is more attractive than muscalure, and that a 7-component synthetic vinegar blend (acetic acid, furfural, 2-phenylethanol, butanoic acid, hexanoic acid, isovaleric acid, and *p*-cresol) is as attractive as vinegar itself. In my study

(Fig. 2.2, Exp. 3), apple cider vinegar, a type of vinegar known to attract *Drosophila* spp. (Landolt *et al.,* 2012), had no effect on attraction of house flies. Similarly, the variety of apple cultivars, and their specific volatile profiles (Dixon & Hewett, 2000), may have contrasting effects on the response of house flies. Smallegange (2003) reports attraction of house flies to apples (variety not specified) but the Honeycrisp apples I bioassay had no such effect (Fig. 2.2, Exp. 6).

The search for food-based fly attractants relies on the assumption that target flies are hungry and food-foraging. This implies that food baits may attract flies only in the absence of alternative food resources (Geden *et al.,* 2009). The type of food attractants tested in my study would not likely be effective at trapping house flies in agricultural settings where manure and rotting organic materials are plentiful. Similarly, in urban environments fly traps are placed near, and will "compete with", dumpsters or garbage bins full of rotting materials. Starving flies, such as the ones I bioassayed, tend to respond more readily than well-fed flies (Smallegange, 2003; Geden *et al.,* 2009). Well-fed flies are simply less motivated to forage and respond less strongly to odorants of human food. This phenomenon highlights the fundamental challenge associated with food-based attractants, a challenge that is addressed only by offering foraging cues that are based on sensory modalities other than, or in addition to, olfaction.

Visual cues may be effective at attracting foraging house flies. Muscid flies have fewer olfactory sensory pits on their antennae than do other filth flies (Bay & Pitts, 1976; Sukontason *et al.,* 2004). Skidmore (1985) argues that house flies may have a relatively diminished sense of smell because they possess many fewer olfactory sensilla on their distal antennal segment compared to other muscid and calliphorid flies. This "numerical deficiency" of olfactory antennal sensilla may possibly be offset by an abundance of contact chemoreceptors on tarsal segments (Dethier, 1955; Skidmore, 1985; Schnuch & Seebaur, 1995), suggesting that foraging house flies may heavily rely on contact chemoreceptive cues when making feeding decisions. Visual foraging cues for house flies have been studied extensively and may be as important as olfactory cues (Conlon & Bell, 1991; Diclaro *et al.,* 2012).

Future research should focus on the development of baits that integrate both visual and olfactory cues attractive to house flies.

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2.6. References

Adams, T.S. and Holt, G.G. (1986) Effect of pheromone components when applied to different model on male sexual behaviour in the house fly, *Musca domestica*. *Journal of Insect Physiology*, 33, 9-18.

Albarak, A.S. (2009) Comparative studies on house fly, *Musca domestica*, populations in different animal farms in relation to attractants and control at Hail Province, Saudi Arabia. *Pakistan Entomologist*, 31, 142-148.

Bay, D.E. and Pitts, C.W. (1976) Antennal olfactory sensilla of the face fly *Musca autumnalis* De Geer (Diptera: Muscidae). *International Journal of Insect Morphology and Embryology*, 5, 1-16.

Brodie, B.S., Smith, M.A., Lawrence, J. and Gries, G. (2015) Effects of floral scent, color and pollen on foraging decisions and oocyte development of common green bottle flies. *PLoS ONE*, 10:e0145055. doi:10.1371/journal.pone.0145055.

Butler, S.M., Gerry, A.C. and Mullens, B.A. (2007) House fly (Diptera: Muscidae) activity near baits containing (Z)-9-tricosene and efficacy of commercial toxic fly baits on a southern California dairy. *Journal of Economic Entomology*, 100, 1489-1495.

Butler, S.M. and Mullens, B.A. (2010) Adult House Fly (Diptera: Mucidae) Activity and age of females near varying levels of (Z)-9-tricosene on a southern California dairy. *Journal of Economic Entomology*, 103, 1929-1936.

Bylka, W., Matlawska, I. and Frański, R. (2010) Essential oil composition of *Taraxacum officinale*. *Acta Physiologiae Plantarum*, 32: 231-234.

Carlson, D.A. and Hogsette, J.A. (2007) Flybrella: A device to attract and kill house flies. *Journal of Economic Entomology*, 100, 483-487.

Carlson, D.A., Mayer, M.S., Silhacek, D.L., James, J.D., Morton Beroza, B., Bierl, B.A. and Calam, D.H. (1971) Sex attractant pheromone of the house fly: Isolation, identification and synthesis. *Science*, 174, 76-78.

Chapman, J.W., Knapp, J.J., Howse, P.E. and Goulson, D. (1998) An evaluation of (Z)-9-tricosene and food odours for attracting house flies, *Musca domestica*, to baited targets in deep-pit poultry units. *Entomologia Experimentalis et Applicata*, 89, 183-192.

Conlon, D. and Bell, W.J. (1991) The use of visual information by house flies, *Musca domestica* (Diptera: Muscidae), foraging in resource patches. *Journal of Comparative Physiology*, 168, 365-371.

Cossé, A.A. and Baker, T.C. (1996) House flies and pig manure volatiles: Wind tunnel behavioral studies and electrophysiological evaluations. *Journal of Agricultural Entomology*, 13, 301-317.

Dafni, A. (1984) Mimicry and deception in pollination. *Annual Review of Ecology and Systematics*, 15, 259-278.

Dethier, V.G. (1955) The physiology and histology of the contact chemoreceptors of the blowfly. *The Quarterly Review of Biology*, 30, 348-371.

Diclaro, J.W., Cohnstaedt, L.W., Pereira, R.M., Allan, S.A. and Koehler, P.G. (2012) Behavioural and physiological response of *Musca domestica* to colored visual targets. *Journal of Medical Entomology*, 49, 94-100.

Dixon, J. and Hewett, E.W. (2000) Factors affecting apple aroma/flavour volatile concentration: A review. *New Zealand Journal of Crop Horticulture*, 28, 155-173.

Dobson, H.E.M. (2006) Relationship between floral fragrance composition and type of pollinator in *Biology of floral scent*. Ed. Dudareva, N. and Pichersky, E. CRC Press, Boca Raton, FL, Pp. 147-198.

Geden, C.J. (2005) Methods for monitoring outdoor populations of house flies, *Musca domestica* L. (Diptera: Muscidae). *Journal of Vector Ecology*, 30, 244-250.

Geden, C.J., Szumlas, D.E. and Walker, T.W. (2009) Evaluation of commercial and field-expedient baited traps for house flies, *Musca domestica*, L. (Diptera: Muscidae). *Journal of Vector Ecology*, 34: 99-103.

Hanley, M.E., Cruickshanks, K.L., Dunn, D., Stewart-Jones, A. and Goulson, D. (2009) Luring house flies (*Musca domestica*) to traps: Do cuticular hydrocarbons and visual cues increase catch? *Medical and Veterinary Entomology*, 23, 26-33.

Hanley, M.E., Dunn, D.W., Abolins, S.R. and Goulson, D. (2004) Evaluation of (Z)-9-tricosene baited targets for control of the house fly (*Musca domestica*) in outdoor situations. *Journal of Applied Entomology*, 128, 478-482.

Hwang, Y.S., Mulla, M.S. and Axelrod, H. (1978) Attractants for synanthropic flies: Ethanol as attractant for *Fannia canicularis* and other pest flies in poultry ranches. *Journal of Chemical Ecology*, 4, 463-470.

Landolt, P.J., Adams, T. and Rogg, H. (2012) Trapping spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), with combinations of vinegar and wine, and acetic acid and ethanol. *Journal of Applied Entomology*, 136, 148-154.

Larson, B.H.M., Kevan, P.G. and Inouye, D.W. (2001) Flies and flowers: Taxonomic diversity of anthophiles and pollinators. *The Canadian Entomologist,* 133, 439-465.

Laor, Y., Koziel, J.A., Cai, L. and Ravid, U. (2015) Chemical-sensory characterization of dairy manure odor using headspace solid-phase microextraction and multidimensional gas chromatography mass spectrometry-olfactometry. *Journal of the Air and Waste Management Association*, 58, 1187-1197.

Mulla, M.S., Hwang, Y.S. and Axelrod, H. (1977) Attractants for synanthropic flies: Chemical attractants for domestic flies. *Journal of Economic Entomology*, 70, 644-648.

Muñoz, A.A. and Cavieres, L.A. (2008) The presence of a showy invasive plant disrupts pollinator service and reproductive output in native alpine species only at high densities. *Journal of Ecology*, 96, 459-467.

Pavia, D.L., Lampman, G.M., Kriz, G.S. and Engel, R.G. (1999a) Extractions, separations, and drying agents in *Introduction to Organic Laboratory Techniques – A Micro Scale Approach*, 3rd Edition. Saunders College Publishing, Orlando, FL. Pp. 615-617.

Pavia, D.L., Lampman, G.M., Kriz, G.S. and Engel, R.G. (1999b) *Simple distillation in Introduction to Organic Laboratory Techniques – A Micro Scale Approach*, 3rd Edition. Saunders College Publishing, Orlando, FL. Pp. 629.

Piasenzotto, L., Gracco, L. and Conte, L. (2003) Solid phase microextraction (SPME) applied to honey quality control. *Journal of the Science of Food and Agriculture*, 83: 1037-1044.

Pickens, L.G., Schmidtmann, E.T. and Miller, R.W. (1994) How to control house and stable flies without using pesticides. US Department of Agriculture, Agriculture Information Bulletin No. 673, p. 14.

Qian, K., Zhu, J.J., Sims, S.R., Taylor, D.B. and Zeng, X. (2013) Identification of volatile compounds from a food-grade vinegar attractive to house flies (Diptera: Muscidae). *Journal of Economic Entomology*, 106, 979-987.

Quinn, B.P., Bernier, U.R., Geden, C.J., Higsette, J.A. and Carlson, D.A. (2007) Analysis of extracted and volatile components in blackstrap molasses feed as candidate house flu attractants. *Journal of Chromatography A*, 1139, 279-284.

Raguso, R.A. (2004) Why do flowers smell? The chemical ecology of fragrance-driven pollination in *Advances in Chemical Ecology*, ed. Cardé, R.T. and Millar, J.G. Cambridge University Press. p. 154.

Schnuch, M. and Seebaur, H. (1998) Sugar cell response to lactose and sucrose in labellar and tarsal taste hairs of *Musca domestica*. *Journal of Comparative Physiology*, 182: 767-775.

Skidmore, P. (1985) The Biology of the Muscidae of the World. Dr. W. Junk Publishers, Dordrecht, The Netherlands. P. 234.

Smallegange, R.C. (2003) Attractiveness of different light wavelengths, flicker frequencies and odours to the house fly (*Musca domestica* L.). Ph.D. Thesis. University of Groningen, The Netherlands.

Sukontason, K., Sukontason, K.L., Piangjai, S., Boonchu, N., Chaiwong, T., Ngern-klun, R., Sripakdee, D., Vogtsberger. and Olson, J.K. (2004) Antennal sensilla of some forensically important flies in families Calliphoridae, Sarcophagidae and Muscidae. *Micron*, 35, 671-679.

Table 2.1 List of objectives (O) and stimuli (S) tested in laboratory experiments for the behavioural responses of responding house flies (RFs) (*Musca domestica*) during 3-h bioassay periods (N = number of replicates)

	Test stimuli (S)	No. RFs	N
O1: Determine the attractiveness of food products and a floral resource to house flies			
Exp. 1	**S1: Diluted dandelion honey** (10 mL); dandelion honey (2): distilled water (1). S2: Distilled water (10 mL).	50	20
Exp. 2	**S1: Dandelion flowers** (2 flowers); two flowers cut and placed in a vial of distilled water. S2: Empty vial.	50	20
Exp. 3	**S1: Apple cider vinegar** (5 mL); pure apple cider vinegar. S2: Empty vial.	50	20
Exp. 4	**S1: Diluted blackstrap molasses** (5 mL); blackstrap molasses (1): distilled water (1). S2: Distilled water (5 mL).	50	10
Exp. 5	**S1: Pale ale beer** (5 mL); flat (air-exposed for 24 h) pale ale beer. S2: Empty vial.	50	10
Exp. 6	**S1: Honeycrisp apples** (3 g); sliced (skinless) Honeycrisp apples. S2: Empty vial.	50	10
O2: Determine whether essential semiochemicals of proven effective sources can be captured in solvent or headspace volatile extracts for attraction of house flies			
Exp. 7	**S1: Headspace volatile extract of dandelion honey.** Vial baited with dandelion honey aeration extract at 196 GHEHs[a] S2: Vial containing a solvent control.	50	22
Exp. 8	**S1: DCM[b] dandelion honey extract.** Vial baited with DCM[b] dandelion honey extract at 11.2 GEEHs[c]. S2: Vial containing a solvent control.	50	20
Exp. 9	**S1: Dandelion honey distillate.** Vial baited with dandelion honey distillate at 16.8 GDEHs[d]. S2: Vial containing a distilled water.	50	10
Exp. 10	**S1: Headspace volatile extract of dandelion flowers (24 FHEs[e]).** Suspended vials within traps were baited with 24 FHE[e] aliquots of headspace volatile extract of dandelion flowers.	50	16

	S2: Solvent control.		
Exp. 11	**S1: Headspace volatile extract of dandelion flowers (240 FHEs[e]).** Suspended vials within traps were baited with 240 FHE[e] aliquots of headspace volatile extract of dandelion flowers.	50	20
	S2: Solvent control.		
Exp. 12	**S1: Headspace volatile extract of dandelion flowers (2,400 FHEs[e]).** Suspended vials within traps were baited with 2,400 FHE[e] aliquots of headspace volatile extract of dandelion flowers.	50	10
	S2: Solvent control.		
Exp. 13	**S1: DCM[b] dandelion flower extract.** Suspended vials within traps were baited with 1 FEE[f] aliquot of dandelion flower extract.	50	10
	S2: Solvent control.		
Exp. 14	**S1: Dandelion flower distillate.** Suspended vials within traps were baited with 8 FDEs[g] aliquots of dandelion flower distillate.	50	10
	S2: Distilled water.		

[a] Gram-Hour-Equivalents-of-Honey (GHEHs); 1 GHEH = the amount of volatiles emitted from 1 g of honey during 1 h)

[b] Dichloromethane (DCM)

[c] Gram-Extract-Equivalents-of-Honey (GEEHs); 1 GEEH = the amount of materials including odorants extracted from 1 g of honey in 100 µl of DCM

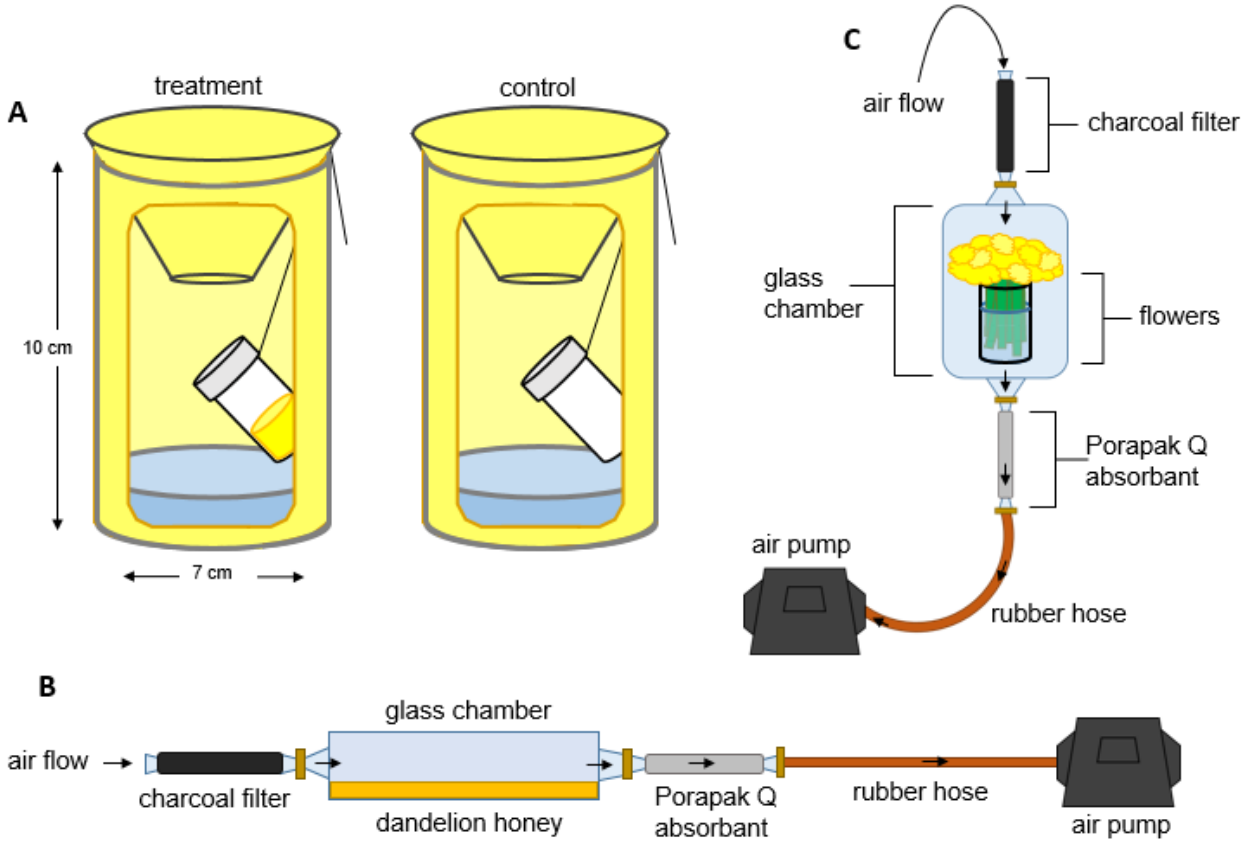
[d] Gram-Distillate-Equivalents-of-Honey (GDEHs); 1 GDEH = the amount of materials including odorants distilled from 1 g of honey present in 100 µl of distillate

[e] Flower-hour-equivalents (FHEs); 1 FHEs = the amount of volatiles emitted from 1 flower in 1 h

[f] Flower-Extract-Equivalent (FEEs); 1 FEE = the amount of materials including odorants extracted from 1 flower in 100 µL of DCM

[g] Flower-Distillate-Equivalents (FDEs); 1 FDE = the amount of materials including odorants distilled from 1 flower in 100 µL of distillate

Figure 2.1 **Graphical illustrations of experimental designs. (A) Paired glass funnel bottle traps, each consisting of a trap base and an inverted trap top (=funnel) covered with yellow paper, and fitted with a wire-suspended vial containing the test or control stimulus under a mesh-covered lid; the drowning fluid at the bottom of the trap ensured captures of responding insects. (B, C) Experimental design to capture odorants emanating from diluted dandelion honey (B) and 100 cut dandelion flowers (C). Odorant-laden air passes through a Porapak-Q odorant trap, where odorants are absorbed and later desorbed with solvent (see methods for detail).**



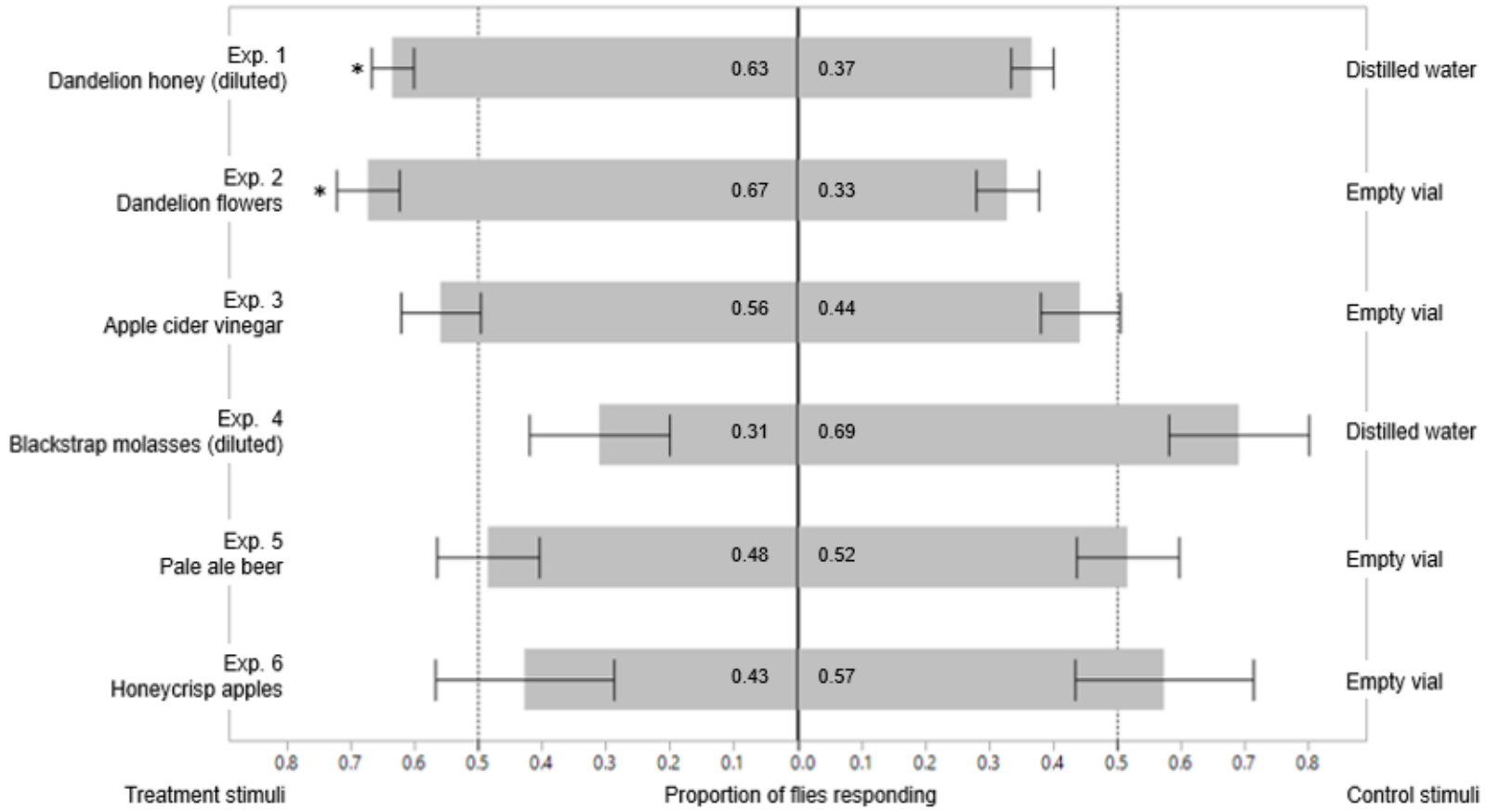


Figure 2.2 **Mean (± SE) proportion of flies responding to various test and control stimuli in experiments 1-6 (see text and Table 2.1 for detail). The asterisks (*) in experiments 1 and 2 denote a statistically significant preference for a test stimulus (two-tailed t-test, Exp. 1: *P* < 0.005, Exp. 2: *P* <0.001).**

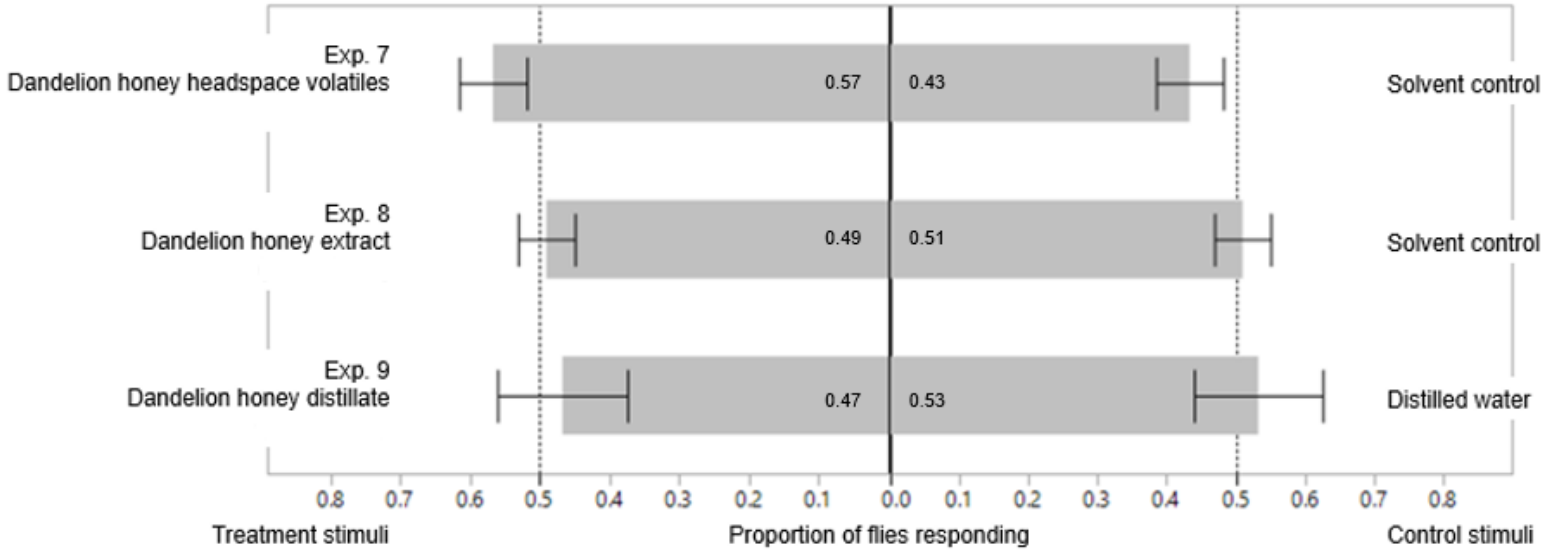


Figure 2.3 **Mean (± SE) proportion of flies responding to (*i*) headspace volatile extract of dandelion honey tested at 196 Gram-Hour-Equivalents-of-Honey (GHEHs; 1 GHEH = the amount of volatiles emitted from 1 g of honey during 1 h) (Exp. 7, N = 22, Table 2.1), (*ii*) dichloromethane (DCM) liquid-liquid dandelion honey extract tested at 11.2 Gram-Extract-Equivalents-of-Honey (GEEHs; 1 GEEH = the amount of materials including odorants extracted from 1 g of honey in 100 µl of DCM) (Exp. 8, N = 20, Table 2.1), and (*iii*) dandelion honey distillate tested at 16.8 Gram-Distillate-Equivalents-of-Honey (GDEHs; 1 GDEH = the amount of materials including odorants distilled from 1 g of honey present in 100 µl of distillate) (Exp. 9, N = 10, Table 2.1).**

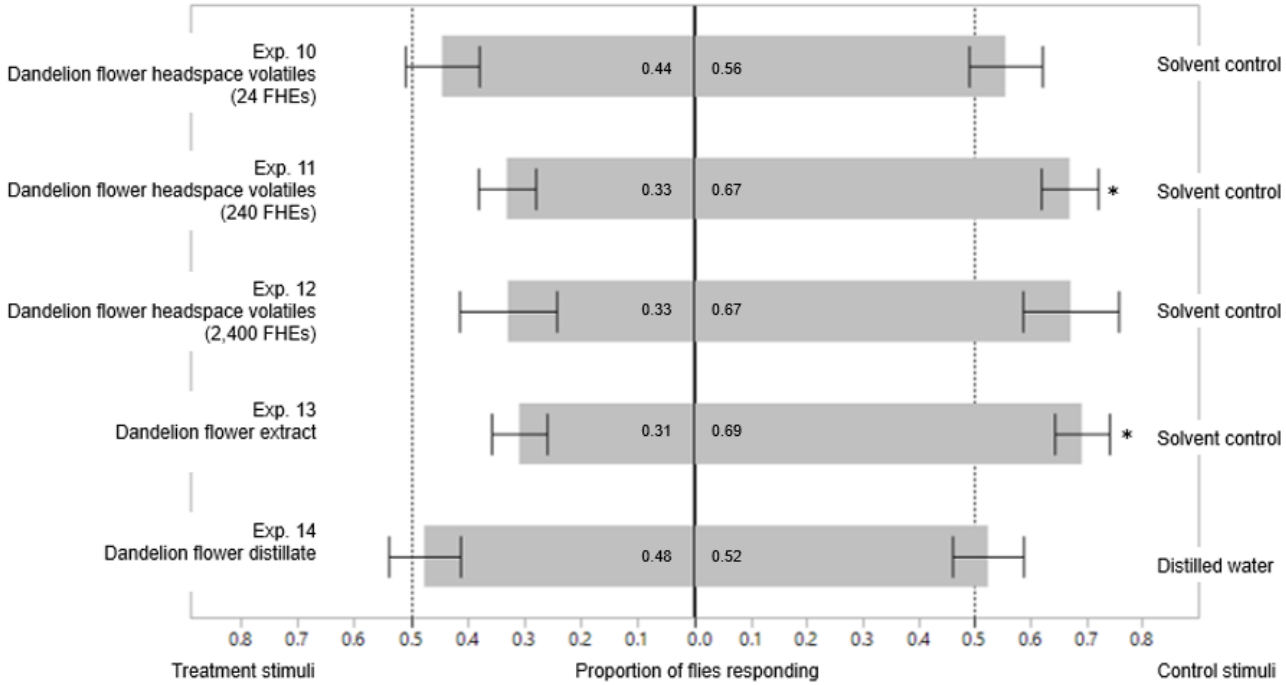


Figure 2.4 **Mean (± SE) proportion of flies responding to (*i*) headspace volatile extract of dandelion flower at 24 Floral-Hour-Equivalents (FHEs; 1 FHE = the amount of volatiles emitted from 1 flower in 1 h) (Exp. 10, N = 16, Table 2.1), (*ii*) 240 FHEs (Exp. 11, N = 20, Table 2.1), (*iii*) and 2,400 FHEs (Exp. 12, N = 10, Table 2.1), (*iv*) DCM dandelion flower extract tested at 1 Flower-Extract-Equivalent (FEE; 1 FEE = the amount of materials including odorants extracted from 1 flower in 100 µL of DCM) (Exp. 13, N = 10, Table 2.1), and (*v*) dandelion flower distillate tested at 0.4 Flower-Distillate-Equivalents (FDEs; 1 FDE = the amount of materials including odorants distilled from 1 flower in 100 µL of distillate) (Exp. 14, N = 10, Table 2.1). The asterisk (*) in experiments 11 and 13 denotes a statistically significant preference for a control stimulus (two-tailed t-test, Exp. 11: $P < 0.01$, Exp. 13: $P < 0.005$).

Chapter 3.

Studying the "fly factor" phenomenon and its underlying mechanisms in house flies, *Musca domestica*[1]

[1]The corresponding manuscript has been resubmitted to Insect Science after peer review, receiving only minor revisions, with authors as follows: Holl, M. and Gries, G.

3.1. Abstract

The "fly factor" was first discovered >60 years ago and describes the phenomenon that food currently or previously fed on by flies attracts more foraging flies than the same type and amount of food kept inaccessible to flies. Since then, there has been little progress made to understanding this phenomenon. Our objectives were (*i*) to demonstrate the existence of the fly factor in house flies, *Musca domestica*, and (*ii*) to study underlying mechanisms that may cause or contribute to the fly factor. In two-choice laboratory bioassays, we obtained unambiguous evidence for a fly factor phenomenon in house flies, in that we demonstrated that feeding flies are more attractive to foraging flies than are non-feeding flies, and that fed-on food is more attractive to foraging flies than is "clean" food. Of the potential mechanisms [fly excreta, metabolic output parameters (elevated temperature, relative humidity, carbon dioxide)], causing the fly factor, fly feces and regurgitate do attract foraging flies but none of the metabolic output parameters of feeding flies does. Even though feeding flies produce significantly more CO_2 than non-feeding flies, elevated levels of CO_2 have no behaviour-modifying effect on flies. Preferential attraction of house flies to fly feces and regurgitate indicates that the flies sense airborne semiochemicals emanating from these sources. Hypothesizing that these semiochemicals are microbe-produced, future studies will aim

at isolating and mass producing these microbes to accumulate semiochemicals for identification.

Key words *Musca domestica*, fly factor, metabolic output, foraging, microbes, semiochemical attractants

3.2. Introduction

The “fly factor” studied here refers to the phenomenon that food currently or previously fed on by flies attracts more foraging flies than the same type and amount of food kept inaccessible to flies (Barnhart & Chadwick, 1953). The feeding activity by flies apparently enhances the recruitment and aggregation of conspecific flies to a food patch (Danchin & Wagner, 1997; Lihoreau & Rivault, 2011). Barnhart & Chadwick (1953) first described the fly factor after their field observations that food batches previously subjected to feeding flies accrue more foraging flies than “fresh” batches. They hypothesized that feeding flies produce and deposit a substance that attracts foraging flies but at the time made no attempt to identify the substance causing the attractiveness of fed-on food (Barnhart & Chadwick, 1953). Subsequent laboratory studies added to the fly factor phenomenon. It became apparent that foraging flies even in the absence of any visual cues prefer sucrose that is currently, or was previously, fed on by flies (Dethier, 1955; Acree *et al.,* 1959). Dethier (1955) also noted that glass surfaces soiled by regurgitate and feces of flies were more attractive to foraging flies than clean surfaces and that the attractiveness was short-lived unless the soiling process continued.

The fly factor phenomenon has been observed in various taxa, including house flies, *Musca domestica* L. (Barnhart & Chadwick, 1953; Acree *et al.,* 1959), black blow flies, *Phormia regina* (Meigen) (Dethier, 1955), green bottle flies, *Lucilia sericata* (Meigen) (Brodie *et al.,* 2015) and face flies, *Musca autumnalis* (DeGreer) (Teskey 1969). The fly factor seems not only widespread but also effective across species. For example, feeding house flies attract foraging black blow flies, and *vice versa* (Dethier, 1955), and feeding black blow flies attract foraging green bottle flies, and *vice versa* (Brodie *et al*., 2015). Despite rigorous attempts, the underlying mechanism(s) of the fly factor remain unknown (Dethier, 1955; Acree *et al.,* 1959).

Reminiscent of the fly factor phenomenon is an incident reported for vinegar flies, *Drosophila melanogaster* (Meigen). Foraging vinegar flies are attracted to food or oviposition resources that have previously been visited by conspecific flies which had – coincidentally – vectored baker's yeast, *Saccharomyces cerevisiae,* to these resources (Becher *et al.,* 2012). The yeast, in turn, produce fermentation semiochemicals that attract foraging vinegar flies. A five-component synthetic blend of the compounds produced by *S. cerevisiae* is as effective as fermenting yeast itself in attracting vinegar flies (Becher *et al.,* 2012).

It is feasible that the fly factor of muscid and calliphorid flies is mediated by metabolic by-products (temperature, relative humidity, CO_2) from well-fed or actively feeding flies. Well-fed insects are often more active and have elevated metabolic functions (McEvoy, 1984; Bradley *et al.,* 2003). Resource-seeking insects could use metabolic indicators of feeding insects to locate essential resources, provided that these indicators sufficiently differ between feeding and non-feeding insects. Honeybees, *Apis mellifera* L., exhibit elevated thoracic temperature after feeding on sugar water with high sugar content (Schmaranzer & Stabentheiner, 1988). Similarly, a forager bee returning from sugar-rich floral resources literally becomes a "hot" waggle dancer when she informs her nest mates about the location of these resources (Stabentheiner & Hagmüller, 1991). Relative humidity as another indicator of previous or current feeding activity was proposed to be the source of the fly factor by both Dethier (1955) and Acree *et al.* (1959). Various taxa of flies are known to concentrate ingested food by extruding liquefied food "bubbles", thus facilitating water evaporation of up to 66% of the weight of the ingested food (Hendrichs *et al.,* 1992; Stoffolano *et al.,* 2008). Accumulative evaporative water loss and an ever increasing relative humidity around feeding flies could then indeed delineate a profitable food source to foraging flies. Carbon dioxide could be a third metabolic indicator of actively feeding or well-fed insects, and their food resource. For example, after a blood meal, kissing bugs, *Rhodnius prolixus* (Stål), emit relatively more CO_2 (Bradley *et al.,* 2003).

Our overall objective was to investigate the fly factor phenomenon in house flies. Our first two specific objectives were to demonstrate unambiguously that the fly factor exists by comparing the attractiveness of food that was being fed on or not, or previously

fed on or not by house flies. With unambiguous evidence for a fly factor, objectives 3-6 were then to determine the underlying mechanisms causing or contributing to the fly factor. Specifically, we investigated the effect of fly feces and regurgitate on attraction of flies, compared the relative attractiveness of fly feces and regurgitate to that of food previously fed on by flies, determined whether volatiles emitted by feeding or non-feeding flies preferentially attract foraging flies, and studied metabolic output (temperature, relative humidity, CO_2) from feeding and non-feeding flies and its effect on attracting foraging flies.

3.3. Materials and methods

3.3.1. Experimental insects

The adult house flies that were tested in experiments over the course of three years originated either from a laboratory-reared strain purchased from a supplier (Beneficial Insectary Inc., Redding, CA, USA) or from maggots collected at a local chicken production facility in Abbotsford, BC, Canada. Flies were reared in the insectary at Simon Fraser University (Burnaby, BC, Canada) at 25 °C, 60% RH, and a photoperiod of L16:D8. Adult flies were provisioned with milk powder, table sugar, and water *ad libitum*. Larvae were reared in 4-L glass jars containing a mixture of wheat bran (2 L), molasses (40 mL), water (700 mL), dry brewer's yeast (20 mL), and thick milk powder paste (50 mL). Bioassays were run with 3- to 5-day-old adult flies that were starved for 16 h prior to bioassays. Although purchased and wild-type flies responded similarly to identical test stimuli, we ran most experiments with wild type flies.

3.3.2. General bioassay design

Two-choice laboratory bioassays were run in six 3-chambered Plexiglas® arenas (Fig. 3.1A) illuminated from above by fluorescent lights (Philips F32TA, Amsterdam, The Netherlands). Dividing walls in each arena contained an inverted metal mesh funnel allowing flies to enter, but not to return from, the lateral arena chambers. The dividing walls were covered in yellow paper (color: canary, Hammermill International Paper Company, item #103341; Memphis, TN, USA) to obscure visual cues associated with

test stimuli. For each replicate, flies were briefly (5 min) cold-sedated and sorted into mixed-sex groups of 100 flies which were then given 10 min to warm to room temperature and subsequently released into the central chamber of the arena. These flies, termed here “foraging flies” (FFs), were given a choice between test stimuli randomly assigned to the lateral chambers of the arena. Test stimuli varied between experiments (see below). Flies that contributed to a test stimulus are termed here “stimulus flies” (SFs). After 2 h, experimental replicates were terminated, the arenas frozen (-20 °C) overnight, and male and female FFs in each lateral chamber, as well as non-responding FFs in the central chamber, were counted the next morning. After each replicate, bioassay arenas were washed with hot water and soap (Sparkleen™, Fisher Science Company, Catalog No. 04-320-4; Pittsburgh, PA) and the yellow paper lining was replaced.

3.3.3. Objective 1: Compare the attractiveness of food being fed on, or not, by flies

Experiment 1 (N = 20, Table 3.1) was designed to test whether a food source being fed on by flies is more attractive to FFs than the same food source in presence of non-feeding flies. The treatment stimulus consisted of a mesh-covered Petri dish (150 × 25 mm; Falcon®, item #353025) containing (*i*) a moist cotton wick (25 × 10 mm), (*ii*) sucrose (15 g) being fed on by 50 SFs which had been starved for 16 h, and (*iii*) a mesh-covered small Petri dish (90 × 25 mm) housing another moist cotton wick (Fig. 3.1A, Exp. 1). The small Petri dish was present only to make the treatment stimulus comparable to the control stimulus. The control stimulus consisted of an identical mesh-covered Petri dish containing (*i*) sucrose (15 g) and (*ii*) a mesh-covered small Petri dish (90 × 25 mm) housing 50 SFs provisioned with a moist cotton wick but not with sucrose (Fig. 3.1A, Exp. 1). The bioassay proceeded as described under “general bioassay design”.

3.3.4. Objective 2: Compare the attractiveness of food previously fed on, or not, by flies

Experiment 2 (N = 30, Table 3.1) was designed to test whether food previously fed on by SFs is more attractive to FFs than food not previously fed on by flies. The

treatment stimulus consisted of a mesh-covered Petri dish (150 × 25 mm) containing a moist cotton wick and sucrose (15 g) previously fed on for 2 h by 50 SFs that were removed just prior to the onset of bioassays (Fig. 3.1A, Exp. 2). The control stimulus consisted of an identical, mesh-covered Petri dish containing a moist cotton wick and sucrose (15 g) (Fig. 3.1A, Exp. 2). The bioassay proceeded as described under “general bioassay design”.

3.3.5. Objective 3: Determine the effect of fly feces and regurgitate on attraction of flies

Experiment 3 (N = 28, Table 3.1) was designed to test whether fly feces and regurgitate attract FFs. The treatment stimulus consisted of a mesh-covered Petri dish (150 × 25 mm) soiled with feces and regurgitate from 50 previously well-fed SFs that had been enclosed in that Petri dish for 2 h with access to a moist cotton wick but not to food (Fig. 3.1A, Exp. 3). The cotton wick remained part of the test stimulus but the SFs were removed from the Petri dish just prior to the onset of bioassays. The control stimulus was identical to the treatment stimulus except that the Petri dish was never exposed to SFs (Fig. 3.1A, Exp. 3). The bioassay proceeded as described under “general bioassay design”.

3.3.6. Objective 4: Compare the attractiveness of fly feces and regurgitate to that of food previously fed on by flies

With evidence that fly feces and regurgitate as well as food previously fed on by SFs are attractive to FFs (see Results), experiment 4 (N = 20, Table 3.1) then was designed to determine whether fed-on food or fly feces/regurgitate are most attractive. Treatment stimulus 1 consisted of a mesh-covered Petri dish (150 × 25 mm) containing a moist cotton wick and sucrose (15 g) previously fed on for 2 h by 50 SFs that were removed just prior to the onset of bioassays (Fig 3.1A, Exp. 4). Treatment stimulus 2 consisted of an identical, mesh-covered Petri dish soiled with feces and regurgitate from 50 previously well-fed SFs that had been enclosed in that Petri dish for 2 h with access to a moist cotton wick but not to food (Fig 3.1A, Exp. 4). The bioassay proceeded as described under “general bioassay design”.

3.3.7. Objective 5: Capture volatiles emitted by feeding flies, or non-feeding flies, and determine their effect on attraction of foraging flies

A set of eight mesh-covered Petri dishes (150 × 25 mm), each containing a moist cotton wick and sucrose (15 g) being fed on by 100 SFs which had been starved for 16 h to ensure feeding, was placed into a Pyrex® glass chamber (340 mm high × 125 mm wide) (Fig. 3.1B). Another set of eight identical, mesh covered Petri dishes, each containing a moist cotton wick and 100 SFs without access to the sucrose (15 g), was placed into a second Pyrex® glass chamber of identical size (Fig. 3.1B). Charcoal-filtered air was drawn at 0.4 L/min for 2 h through each chamber and a glass tubing (5 i.d. × 120 mm) containing 6 g of the adsorbant Porapak-Q (50-80 mesh, Waters Associates Inc. Milford, MA, USA). Volatiles were desorbed (extracted) from the Porapak-Q with 2 mL of pentane. This process was repeated multiple times, accumulating volatiles from approximately 3,000 flies that were either feeding or non-feeding for 2 h. Thus, each of the two resulting Porapak-Q headspace volatile extracts contained a total of 6,000 fly-hour-equivalents (FHEs) of volatile emission (2 FHEs = the amount of volatiles emitted from one fly during 2 h of feeding or non-feeding). Extracts were concentrated to 4 FHE/µL.

Experiment 5 (N = 12, Table 3.1) tested whether headspace volatile extract of feeding SFs is more attractive to FFs than headspace volatile extract of non-feeding SFs. For each two-choice bioassay, a test stimulus was prepared by (*i*) placing a piece of filter paper (one quarter of a Whatman™ 55-mm diam disc, cat. no. 1001-055) in a Pyrex Petri dish (50 × 15 mm), (*ii*) impregnating filter paper with a 400-FHE aliquot of one of the two headspace volatile extracts, and (*iii*) randomly assigning each Petri dish to a lateral chamber of the test arena (Fig. 3.1A). The bioassay proceeded as described under “general bioassay design”.

3.3.8. Objective 6: Determine the metabolic output (temperature, relative humidity, CO_2) from feeding and non-feeding flies and its effect on attracting foraging flies

Metabolic outputs from groups of feeding or non-feeding flies were recorded with an indoor-air-quality-monitor (Q-TRAK model 7575, TSI Inc., Shoreview, MN, USA). For each recording (N = 8), a mesh-covered Petri dish (150 × 25 mm) with 50 SFs feeding on sucrose (15 g) (N = 4), or 50 non-feeding SFs unable to access the sucrose (N = 4), was placed into a lateral chamber of a bioassay arena. The air quality probe was inserted into a chamber through a tight-fitting hole (20 mm diameter) (Fig. 3.1C), recording for 2 h concurrently the temperature (°C), relative humidity (%), and CO_2 concentration (ppm) near a group of flies. The monitor logged data every minute as an average of the previous 60 s of data recordings.

With evidence that the CO_2 output of feeding SFs is significantly higher than that of non-feeding SFs (see results), and thus could provide a cue for FFs, experiment 6 (N = 16, Table 3.1) was designed to test the effect of CO_2 on the response of FFs. To simulate the CO_2 concentration associated with feeding SFs, the equivalent amount of CO_2 (prepared as 1% CO_2 in breathing air, Praxair Canada Inc., Mississauga, ON, Canada) was gently pumped at 30 ml per minute through copper tubing (1.5 m × 2 mm i.d.) interconnected to an aluminum tubing (0.5 m × 0.5 mm i.d) that directed the gas mixture into the treatment chamber of the bioassay arena (Fig. 3.1D). Breathing air (Praxair) without additional CO_2 was pumped in a similar way into the control chamber (Fig. 3.1D). Both the treatment and control chamber also contained a Petri dish with fed-on sucrose and a moist cotton wick (see test stimulus of experiment 1) to address the possibility that a CO_2 effect might express itself only in the presence of semiochemicals emanating from fly deposits. The bioassay proceeded as described under “general bioassay design”.

3.3.9. Statistical analyses

For each of experiments 1-6, the mean proportion of FFs responding to a treatment stimulus was compared by one-sample t-test to an equal (0.5) proportion of responders to each of the two test stimuli. Moreover, the FFs that remained in the central

chamber at the end of bioassays are reported as the mean proportion of all FFs released that did not respond to either test stimulus (mean proportion of non-responders).

Metabolic output parameters of flies (temperature, relative humidity, CO_2) were analysed separately for differences between the four groups of feeding flies and the four groups of non-feeding flies. Temperature measurements were analysed using an ANCOVA to determine a possible interaction between temperature over time and the activity of flies (feeding or non-feeding). Humidity measurements were fitted to an exponential growth model [$y = a(1 - be^{-kx})$] characterised by the horizontal asymptote (*a*), scale (*b*), and growth rate (*k*). The model was fitted to each replicate and the means of *a*, *b*, and *k* from feeding and non-feeding groups of flies were compared using a two-tailed t-test to identify any differences in the humidity outputs between groups over time. CO_2 outputs of flies over time were measured as differentials relative to ambient CO_2 levels. Ambient CO_2 levels, in turn, were determined by averaging CO_2 readings over 10 min from empty chambers just prior to the onset of metabolic output recordings of feeding or non-feeding flies. The mean ambient CO_2 level was then subtracted from the mean CO_2 output of feeding or non-feeding flies. An ANCOVA was run to determine whether there was an interaction between CO_2 output over time and the activity of flies (feeding or non-feeding). All data were analyzed by JMP 12 (SAS Institute Inc.).

3.4. Results

3.4.1. Objective 1: Compare the attractiveness of food being fed on, or not, by flies

The proportion (mean ± SE) of FFs responding to SFs feeding on sucrose (0.66 ± 0.03) was significantly greater than the proportion of FFs responding to SFs unable to access the sucrose ($t = 5.0398$, $P < 0.0001$; Fig. 3.2, Exp. 1), indicating that the activity of feeding enhances the attractiveness of a food source. The mean proportion (± SE) of FFs not responding to either test stimulus was 0.44 (± 0.03).

3.4.2. Objective 2: Compare the attractiveness of food previously fed on, or not, by flies

The proportion (mean ± SE) of FFs responding to sucrose previously fed on by SFs (0.59 ± 0.12) was significantly greater than the proportion of FFs responding to sucrose not previously fed on by flies (t = 3.8635, $P < 0.001$; Fig. 3.2, Exp. 2), indicating that even previous feeding activity by SFs on a food source still enhanced its attractiveness. The mean proportion (± SE) of FFs not responding to either test stimulus was 0.42 (± 0.02).

3.4.3. Objective 3: Determine the effect of fly feces and regurgitate on attraction of flies

The proportion (mean ± SE) of FFs responding to a Petri dish soiled with fly feces and regurgitate (0.56 ± 0.13) was significantly greater than the proportion of FFs responding to a clean Petri dish (t = 2.5830, $P < 0.05$; Fig. 3.2, Exp. 3), indicating that fly feces and/or regurgitate release semiochemicals that attract FFs. The mean proportion (± SE) of FFs not responding to either test stimulus was 0.49 (± 0.02).

3.4.4. Objective 4: Compare the attractiveness of fly feces and regurgitate to that of food previously fed on to by flies

The proportion (mean ± SE) of FFs responding to a Petri dish soiled with fly feces and regurgitate (0.48 ± 0.03) did not differ statistically from the proportion of FFs responding to a Petri dish containing sucrose previously fed on by SFs (t = 0.5116, $P = 0.6690$; Fig. 3.2, Exp. 4), indicating that both sources release the same semiochemicals that attract FFs, or that these sources release different semiochemicals that are equally attractive to FFs. The mean proportion (± SE) of FFs not responding to either test stimulus was 0.37 (± 0.03).

3.4.5. Objective 5: Capture volatiles emitted by feeding flies, or non-feeding flies, and determine their effect on attracting foraging flies

The proportion (mean ± SE) of FFs responding to headspace volatile extract of SFs feeding on sucrose (0.45 ± 0.04) did not differ statistically from the proportion of FFs responding to headspace volatile extract of SFs that had no access to sucrose (t = 1.1461, *P* = 0.2761). The mean proportion (± SE) of FFs not responding to either test stimulus was 0.41 (± 0.04).

3.4.6. Objective 6: Determine the metabolic output (temperature, relative humidity, CO_2) from feeding and non-feeding flies and its effect on attracting foraging flies

There was no covariant interaction between temperature over time and the activity of flies (feeding or non-feeding) (F = 0.0587, *P* = 0.8086; Fig. 3.3A). There was also no effect of time on temperature irrespective of flies feeding or not (F = 0.4512, *P* = 0.5019). Finally, there were no temperature differentials between feeding and non-feeding flies regardless of time (F = 0.4379, *P* = 0.5085).

The humidity outputs of flies were fitted to the model $y = a(1 - be^{-kx})$. Comparing the mean (± SE) of each variable (*a*, *b*, *k*) from feeding flies [*a* = 75.06 (1.3), *b* = 0.47 (0.1), *k* = 0.04 (0.01)] and from non-feeding flies [*a* = 73.10 (3.7), *b* = 0.47 (0.1), *k* = 0.05 (0.00)] revealed no statistical difference between the two fly groups for *a* (t = -0.9866, *P* = 0.3837), *b* (t = 0.0413, *P* = 0.9689), or *k* (t = 1.6928, *P* = 0.1573), indicating no difference in relative humidity output between feeding and non-feeding flies over time (Fig. 3.3B). However, there was significant covariant interaction between the CO_2 output over time and the activity of flies (feeding or starving) (F = 351.9, *P* < 0.0001).

The proportion (mean ± SE) of FFs selecting the lateral chamber with enhanced CO_2 input (0.46 ± 0.03) did not differ statistically from the proportion of FFs selecting the lateral chamber without enhanced CO_2 input (Exp. 6: t = 1.2712, *P* = 0.2244), indicating that the elevated level of CO_2 associated with feeding flies (Fig. 3.3C) does not attract FFs, and thus does not contribute to the superior attractiveness of feeding flies over non-

feeding flies (see Fig. 3.2, Exp. 1). The mean proportion (± SE) of FFs not responding to either test stimulus was 0.24 (± 0.02).

3.5. Discussion

We present unambiguous evidence for a fly factor phenomenon in house flies, in that we demonstrate that feeding flies are more attractive than non-feeding flies, and that fed-on food is more attractive than "clean" food of the same type and amount. Searching for mechanisms underlying the fly factor, we show that regurgitate and feces of fed flies attract foraging flies. As we could not prove that feeding flies or their excreta produce attractive semiochemicals, we focussed instead on metabolic by-products of feeding flies as potential cues for foraging flies. While neither temperature nor relative humidity differed between feeding and non-feeding flies, feeding flies produced significantly more CO_2 than non-feeding flies, suggesting that CO_2 – at elevated levels – may signify feeding activity and thus the presence of a food source. However, experimental testing of CO_2 as a foraging cue for flies did not reveal any behaviour-modifying effect. These results align with previous findings that house flies are not attracted to CO_2 (Richards, 1922; Wieting & Hoskins, 1939). Based on our data we conclude that relative humidity, temperature, and CO_2 on their own are not causing the fly factor.

Preferential attraction of house flies to fed-on food (Fig. 3.2, Exp. 2), or to house fly feces and regurgitate (Fig. 3.2, Exp. 3), indicates that the flies sense airborne semiochemicals emanating from these sources. That we could not capture the essential semiochemicals on Porapak-Q and demonstrate attractiveness of Porapak-Q headspace volatile extract implies that these semiochemicals are produced at quantities sufficiently high to be detected by flies but too low to be captured, or not lost during Porapak-Q extraction. Visual cues associated with test stimuli did not likely affect the flies' response because these cues hardly differed between test stimuli and would have been apparent only after the flies had entered a mesh funnel leading into a stimulus chamber.

It is conceivable that the semiochemicals associated with current or past feeding activities of flies are produced by symbiotic microbes. The proposed concept that feeding and defecating flies inoculate food resources with symbiotic microbes (Hendrichs *et al.,*

1992) is well supported by recent reports that the digestive tract of house flies sustains a diverse microbial community (Gupta *et al.,* 2012). Furthermore, as the sponging mouthparts of flies are capable of taking up only liquid foods or solid foods after being dissolved in watery regurgitates, any microbes – if present in these regurgitates – may become inocula for food resources. Indeed, salivary glands of bottle fly maggots have recently been shown to contain the bacterium *Proteus mirabilis* (Ma *et al.,* 2012) which produces some of the semiochemicals associated with decaying flesh that attract foraging and ovipositing bottle flies (Ma *et al.,* 2012; Tomberlin *et al.,* 2012). Interestingly, *P. mirabilis* has also been isolated from house fly guts (Gupta *et al.,* 2012) and shown to produce indole and dimethyltrisulfide (Tomberlin *et al.,* 2012) which are known house fly and blow fly attractants (Mulla *et al.,* 1977; Cossé & Baker, 1996, Brodie *et al.,* 2014, 2016).

The hypothesis of a microbe-mediated fly factor is further supported by distinct behavioural responses of flies in two-choice experiments. While flies were attracted to previously fed on sucrose (Fig. 3.2, Exp. 2), and to Petri dishes soiled with fecal and regurgitation deposits of flies (Fig. 3.2, Exp. 3), they were more strongly attracted to flies actively feeding on sucrose (Fig. 3.2, Exp. 1). The explanation for this differential response of bioassayed flies may lie in the specific moisture content of a resource that may, or may not, suffice to support sustained microbial growth and semiochemical emission. Fly feces and regurgitates are sufficiently moist and thus suitable to sustain microbial activity (Olsen 1998; Gupta *et al.,* 2012; Ma *et al.,* 2012), at least for a short while. However, as soon as these deposits desiccate, the sucrose becomes too dry to support microbial populations (Tapia *et al.,* 2007), which may explain Dethier's observation (1955) that fly deposits are attractive for only a short time period. In comparison, actively feeding and thus defecating and regurgitating flies continuously add to the moisture content of a food source which appears to be a key for the well-being of microbes, and likely for their production of semiochemicals.

In light of ever increasing evidence for microbe-mediated communication among insects (e.g., Lam *et al.,* 2007; Davis *et al.,* 2013; Woodbury, 2012; Woodbury & Gries, 2013; Woodbury *et al.*, 2013), and microbial semiochemicals serving as foraging cues for insects (e.g., Becher *et al.*, 2012; Wada-Katsumata *et al.*, 2015), we do anticipate

that the hypothesis of a microbe-mediated fly factor will be supported in future studies. The challenge of identifying the microbial semiochemicals that constitute the fly factor, and that attract foraging flies, could be addressed by greatly enhancing semiochemical production. We intend to accomplish this in follow-up studies by (*i*) allowing flies to feed on, and thus inoculate, diverse types of nutrient media that support microbial growth, (*ii*) bioassaying the response of flies to media with microbial growth, (*iii*) isolating and mass producing those microbes that attract flies, and (*iv*) accumulating the semiochemicals of attractive microbes for chemical analyses.

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3.6. References

Acree, F., Davis, P.L., Spear, S.F., LaBrecque, G.C. and Wilson, H.G. (1959) Nature of the attractant in sucrose fed on by house flies. *Journal of Economic Entomology*, 52, 981–985.

Barnhart, C.S. and Chadwick, L.E. (1953) A “fly factor” in attractant studies. *Science*, 117, 104–105.

Becher, P.G., Flick, G., Rozpędowska, E., Schmidt, A., Hagman, A., Lebreton, S., Larsson, M.C., Hansson, B.S., Piškur, J., Witzgall, P. and Bengtsson, M. (2012) Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Functional Ecology*, 26, 822–828.

Bradley, T.J., Brethorst, L., Robinson, S. and Hetz, S. (2003) Changes in the rate of CO2 release following feeding in the insect *Rhodnius prolixus*. *Physiology and Biochemical Zoology*, 76, 302–309.

Brodie, B.S., Babcock, T., Gries, R. and Gries, G. (2016) Acquired smell? Mature females of the common green bottle fly shift semiochemical preferences from feces feeding sites to carrion oviposition sites. *Journal of Chemical Ecology*, 42, 40–50.

Brodie, B.S., Gries, R., Martins, A., VanLaerhoven, S. and Gries, G. (2014) Bimodal cue complex signifies suitable oviposition sites to gravid females of the common green bottle fly. *Entomologia Experimentalis et Applicata*, 153, 114–127.

Brodie, B.S., Wong, W.H.L., VanLaerhoven, S. and Gries, G. (2015) Is aggregated oviposition by the blow flies *Lucilia sericata* and *Phormia regina* (Diptera: Calliphoridae) really pheromone-mediated? *Insect Science*, 22, 651–660.

Cossé, A.A. and Baker, T.C. (1996) House flies and pig manure volatiles: Wind tunnel behavioral studies and electrophysiological evaluations. *Journal of Agricultural Entomology*, 13, 301-317.

Danchin, E. and Wagner, R.H. (1997) The evolution of coloniality: the emergence of new perspectives. *Trends in Ecology & Evolution*, 12, 342–347.

Davis, T.S., Crippen, T.L., Hofstetter, R.W. and Tomberlin, J.K. (2013) Microbial volatile emissions as insect semiochemicals. *Journal of Chemical Ecology*, 39, 840–859.

Dethier, V.G. (1955) Mode of action of sugar-baited fly traps. *Journal of Economic Entomology*, 48, 235–239.

Gupta, A.K., Nayduch, D., Verma, P., Shah, B., Ghate, H.V., Patole, M.S. and Schouce, Y.S. (2012) Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica* L.). *FEMS Microbial Ecology*, 79, 581–593.

Hendrichs, J., Cooley, S.S. and Prokopy, R.J. (1992) Post-feeding bubbling behaviour in fluid-feeding Diptera: concentration of crop contents by oral evaporative excess water. *Physiological Entomology*, 17, 153–161.

Lam, K., Babor, D., Duthie, B., Babor, E.M., Moore, M. and Gries, G. (2007) Proliferating bacterial symbionts on house fly eggs affects oviposition behaviour of adult flies. *Animal Behaviour*, 74, 81–92.

Lihoreau, M. and Rivault, C. (2011) Local enhancement promotes cockroach feeding aggregations. *PLoS ONE*, 6, 1–7.

Ma, Q., Fonseca, A., Liu, W., Fields, A.T., Pimsler, M.L., Spindola, A.F., Tarone, A.M., Crippen, T.L., Tomberlin, J.K. and Wood, T.K. (2012) *Proteus mirabilis* interkingdom swarming signals attract blow flies. *The ISME Journal*, 6, 1356–1366.

McEvoy, P.B. (1984) Increase in respiratory rate during feeding in larvae of the cinnabar moth *Tyria jacobaeae*. *Physiological Entomology*, 9, 191–195.

Mulla, M.S., Hwang, Y.S. and Axelrod, H. (1977) Attractants for synanthropic flies: Chemical attractants for domestic flies. *Journal of Economic Entomology*, 70, 644-648.

Nicolson, S.W. and Louw, G.N. (1982) Simultaneous measurement of evaporative water loss, oxygen consumption, and thoracic temperature during flight in a carpenter bee. *Journal of Experimental Zoology*, 222, 287–296

Olsen, A.R. (1998) Regulatory action criteria for filth and other extraneous materials: III. Review of flies and foodborne enteric disease. *Regulatory Toxicology and Pharmacology*, 28, 199-211.

Richards, C.H. (1922) Is the house fly in its natural environment attracted to carbon dioxide? *Journal of Economic Entomology*, 15, 425–430.

Schmaranzer, S. and Stabentheiner, A. (1988) Variability of the thermal behaviour of honeybees on a feeding place*. Journal of Comparative Physiological Biology*, 158, 135–141.

Stabentheiner, A. and Hagmüller, K. (1991) Sweet food means “hot dancing” in honeybees. *Naturwissenschaften*, 78, 471–473.

Stoffolano, J.G., Acaron, A. and Conway, M. (2008) “Bubbling” or droplet regurgitation in both sexes of adult *Phormia regina* (Diptera: Calliphoridae) fed various concentrations of sugar and protein solutions. *Annals of the Entomological Society of America*, 101, 964–970.

Tapia, M.S., Alzamora, S.M. and Chirife, J. (2007) Effects of Water Activity (aw) on Microbial Stability: As a Hurdle in Food Preservation. Water Activity in Foods: Fundamentals and Applications (eds. Barbosa-Cánovas, G.V., Fontana Jr., A.J., Schmidt, S.J., and Labuza, T.P.), pp. 239–272. Blackwell Publishing Ltd, Oxford, UK.

Teskey, H.J. (1969) On the behavior and ecology of the face fly, *Musca autumnalis* (Diptera: Muscidae). *The Canadian Entomologist*, 101, 561–576.

Tomberlin, J.K., Crippen, T.L., Tarone, A.M., Singh, B., Adams, K., Rezenom, Y.H., Benbow, M.E., Flores, M., Longnecker, M., Pechal, J.L., Russel, D.H., Beier, R.C. and Wood, T.K. (2012) Interkingdom response of flies to bacteria mediated by fly physiology and bacteria quorum sensing. *Animal Behaviour*, 84, 1449–1456.

Wada-katsumata, A., Zurek, L., Nalyanya, G. and Schal, C. (2015) Gut bacteria mediate aggregation in the German cockroach. *PNAS*, 15678–15683.

Wieting, J.O.G and Hoskins, W.M. (1939) The olfactory response of flies in a new type of insect olfactometer. *Journal of Economic Entomology*, 32, 24–29.

Woodbury, N. (2012) Microorganisms that benefit insects: A guide to their transmission and experimentally demonstrated benefit. LAP Lambert Academic Publishing GmbH & Co. KG, Saarbrücken, Germany.

Woodbury, N. and Gries, G. (2013) Firebrats, *Thermobia domestica*, aggregate in response to the microbes *Enterobacter cloacae* and *Mycotypha microspore*. *Entomologia Experimentalis et Applicata*, 1447, 154–159.

Woodbury, N., Moore, M. and Gries, G. (2013) Horizontal transmission of the microbial symbionts *Enterobacter cloacae* and *Mycotypha microspora* to their firebrat host. *Entomologia Experimentalis et Applicata*, 147, 160–166.

Table 3.1 List of objectives (O) and stimuli (S) tested in laboratory experiments for the behavioural responses of foraging flies (FFs) (*Musca domestica*) during 2-h bioassay periods (N = number of replicates)

Stimuli tested	No. FFs	N
O1: Compare the attractiveness of food being fed on, or not, by flies		
S1: Feeding flies (50 flies feeding on 15 g of sucrose) **S2: Non-feeding flies** (50 flies without access to 15 g of sucrose)	100	20
O2: Compare the attractiveness of food previously fed on, or not, by flies		
S1: Fed-on sucrose (15 g of sucrose previously fed on by 50 flies) **S2: Fresh sucrose** (15 g of sucrose that was not previously fed on)	100	30
O3: Determine the effect of fly feces and regurgitate on attraction of flies		
S1: Soiled Petri dish (Petri dish soiled with feces and regurgitate from 50 well-fed flies during 2 h) **S2: Clean Petri dish** (Clean Petri dish without prior exposure to flies)	100	28
O4: Compare the attractiveness of fly feces and regurgitate to that of food previously fed on by flies		
S1: Fed-on sucrose (15 g of sucrose previously fed on by 50 flies) **S2: Soiled petri dish** (Petri dish soiled with feces and regurgitate from 50 well-fed flies during 2 h)	100	20
O5: Capture volatiles emitted by feeding flies, or non-feeding flies, and determine their effect on attraction of foraging flies		
S1: Headspace volatile extract of feeding flies (Filter paper impregnated with 400 FHE[a] of feeding flies) **S2: Headspace volatile extract of non-feeding flies** (Filter paper impregnated with 400 FHE[a] of non-feeding flies)	100	12
O6: Determine the metabolic output (temperature, relative humidity, CO_2) from feeding and non-feeding flies and its effect on attracting foraging flies		
S1: CO_2-enriched air (Breathing air enriched with 1% CO_2 was pumped into treatment chamber of bioassay arena) **S2: Air** (Breathing air was pumped into control chamber of bioassay arena)	100	15

[a]Fly-hour-equivalent (FHE); 1 FHE = the amount of volatiles emitted from one fly during 1 h of feeding or non-feeding

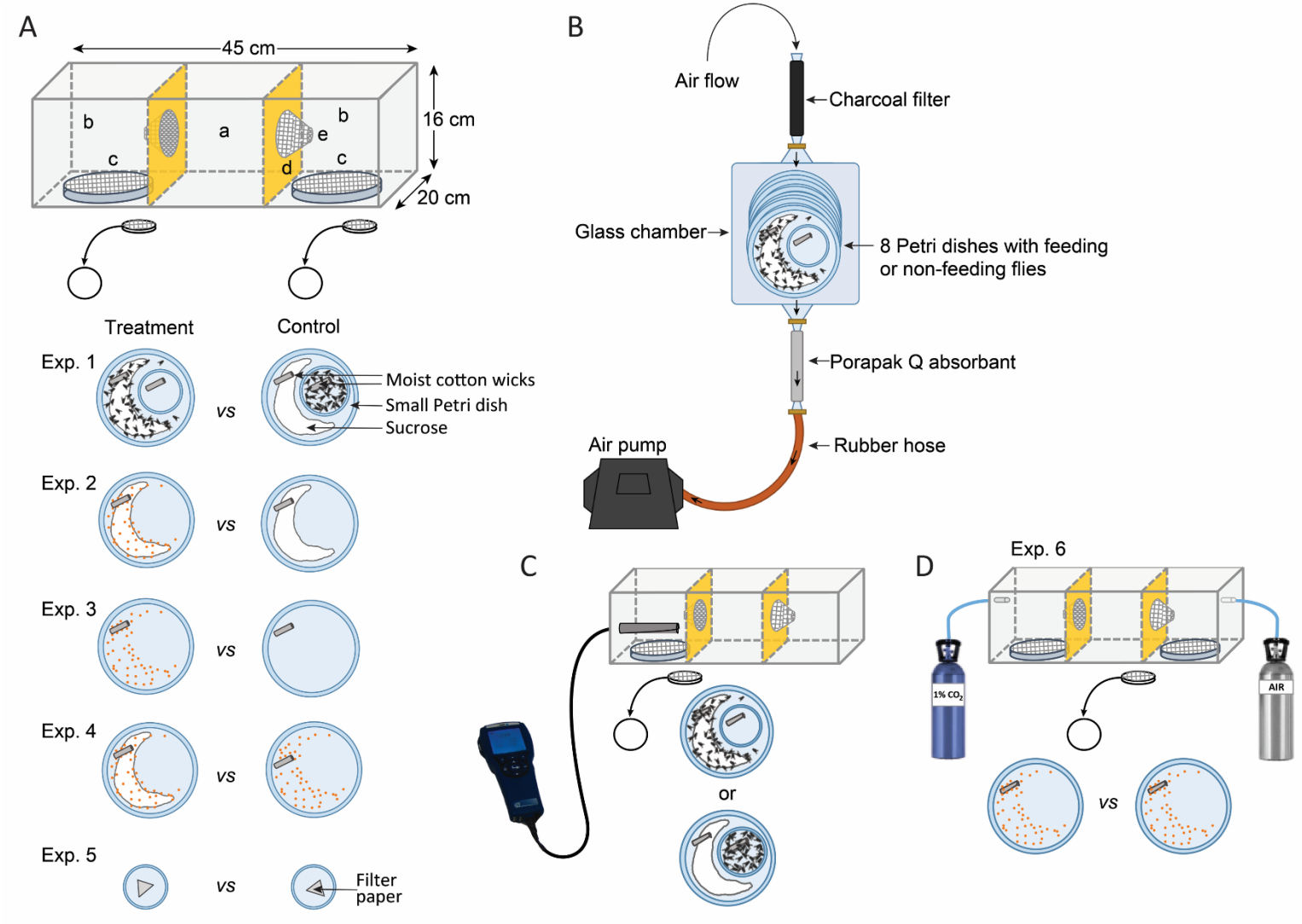
A
45 cm
16 cm
20 cm
b
a
b
c
d
e
c
Treatment
Control
Exp. 1
vs
Moist cotton wicks
Small Petri dish
Sucrose
Exp. 2
vs
Exp. 3
vs
Exp. 4
vs
Exp. 5
vs
Filter paper
B
Air flow
Charcoal filter
Glass chamber
8 Petri dishes with feeding or non-feeding flies
Porapak Q absorbant
Air pump
Rubber hose
C
or
D
Exp. 6
1% CO2
AIR
vs

Figure 3.1 **(A) Graphical illustration of a two-choice Plexiglas® arena consisting of a central chamber (a) for the release of 100 bioassay (foraging) flies, and of two lateral chambers (b) each housing a randomly assigned test stimulus presented in a mesh-covered Petri dish (c). Dividing walls (d) were covered with yellow paper to obscure visual cues associated with test stimuli and were fitted with an inverted metal mesh funnel (e) allowing flies to enter, but not to return from, the lateral chambers. Test stimuli in experiments 1-5 consisted of: (*i*) house flies feeding, or not, on sucrose (Exp. 1); (*ii*) sucrose previously fed on (as indicated by yellow fecal dots), or not, by house flies (Exp. 2); (*iii*) a Petri dish soiled with fly fecal and regurgitation deposits (yellow dots) or kept clean (Exp. 3); (*iv*) sucrose previously fed on or a soiled Petri dish (Exp. 4); (*v*) filter paper treated with aliquots of headspace volatile extract of feeding flies (see B) or a solvent control (Exp. 5) (see methods for detail). (B) Illustration of the experimental design to capture odorants emitted from 100 flies feeding on sucrose in each of eight staggered Petri dishes. Odorant-laden air passed through the Porapak-Q odorant trap, where odorants were absorbed and later desorbed with solvent (see methods for detail). (C) Illustration of a two-choice Plexiglas® arena fitted with an air-quality-monitor-probe (Q-TRAK model 7575) to measure over-time changes of temperature, relative humidity, and carbon dioxide associated with flies feeding, or not, on sucrose (see methods for details). (D) Illustration of a two-choice Plexiglas® arena fitted with tubing for the delivery of CO_2-enriched (1%) breathing air (treatment stimulus) or breathing air (control stimulus). The CO_2 concentration in the treatment chamber resembled that around feeding flies. Petri dishes soiled with fecal and regurgitation deposits of flies were kept in each chamber to address the possibility that a CO_2 effect might express itself only in the presence of semiochemicals emanating from fly deposits.**

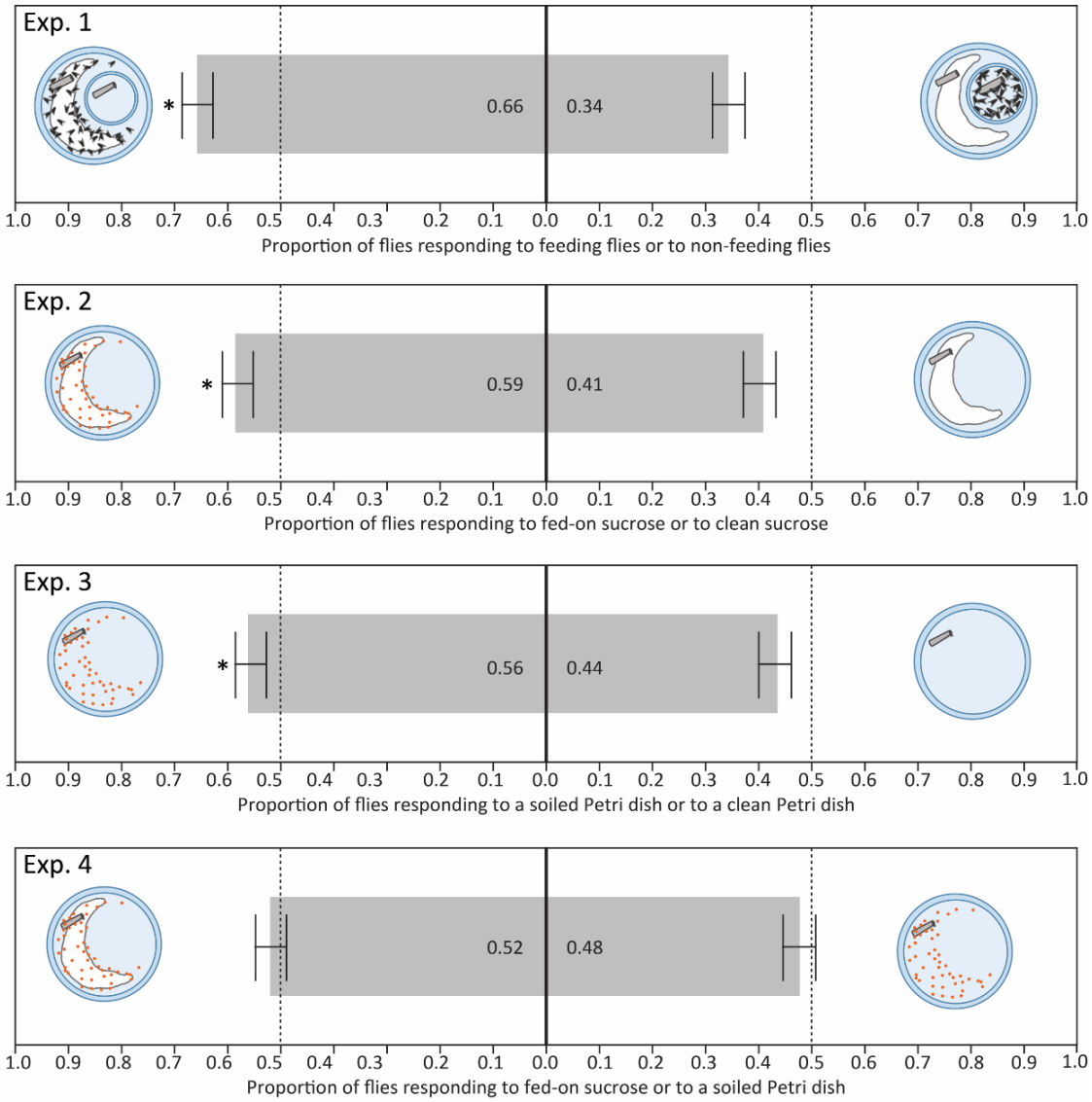

Figure 3.2 **Mean (± SE) proportion of foraging flies responding to (i) stimulus flies feeding on sucrose (left), or not (Exp. 1, N = 20, Table 3.1); (ii) sucrose previously fed on (left), or not, by stimulus flies (Exp. 2, N = 30, Table 3.1); (iii) a Petri dish soiled with fecal and regurgitation deposits by flies (left) and or kept clean (Exp. 3, N = 28, Table 3.1); and (iv) previously fed on sucrose (left) and a soiled Petri dish (Exp. 4, N = 20, Table 3.1). The asterisks (*) in experiments 1, 2 and 3 denote a statistically significant preference for a test stimulus (two-tailed t-test, Exp. 1: P < 0.0001, Exp. 2: P < 0.001, Exp. 3: P <0.05). The mean proportion (± SE) of non-responding foraging flies in experiments 1, 2, 3 and 4 were 0.44 (± 0.03), 0.42 (± 0.02), 0.49 (± 0.02), and 0.37 (± 0.03), respectively.**

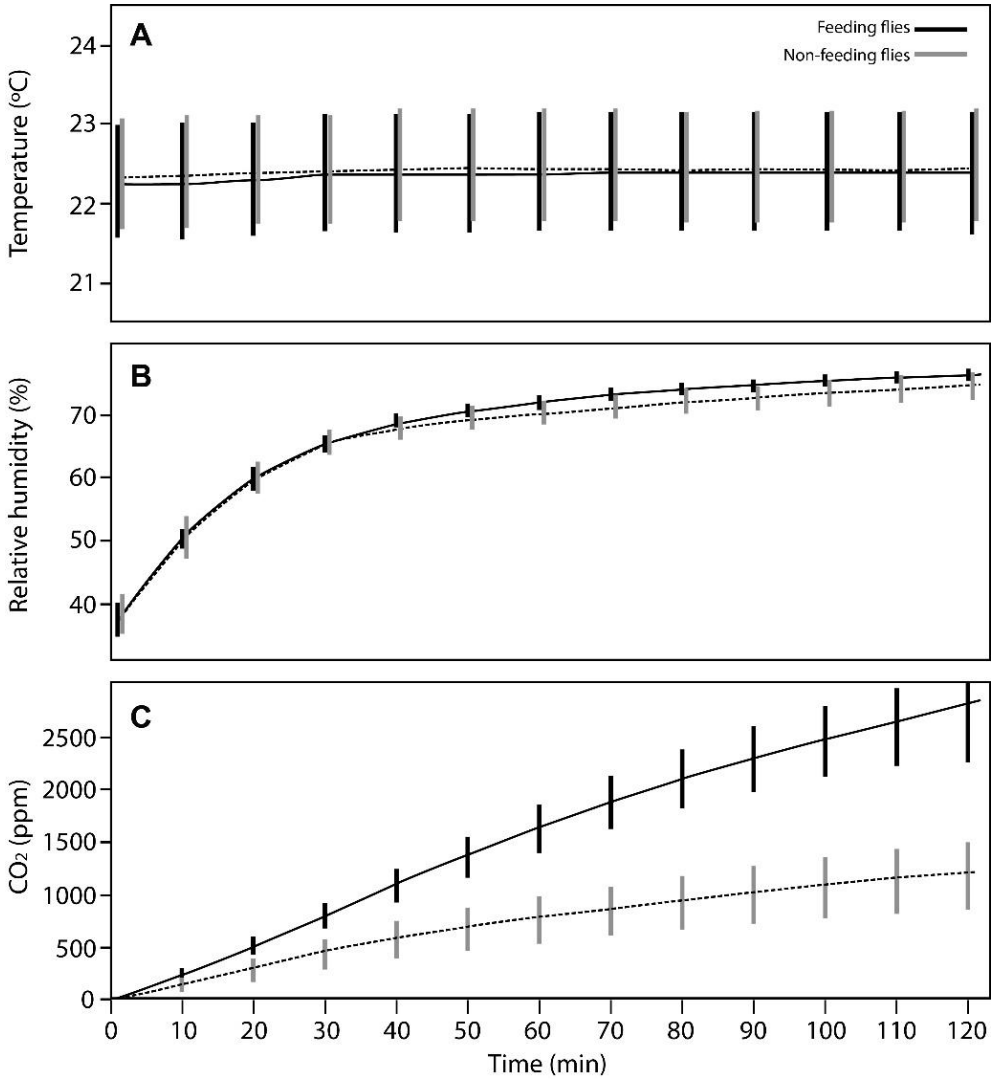

Figure 3.3 **Metabolic output parameters [temperature (A), relative humidity (B), CO_2 (C)] recorded concurrently from groups of feeding flies (solid lines) or non-feeding flies (dashed lines) (N = 4 each). There was no covariant interaction between temperature over time and the activity of flies (feeding or non-feeding) (ANCOVA, *P* = 0.8086). Relative humidity outputs were fitted to the model $y = a(1 - be^{-kx})$. Between the groups of feeding and non-feeding flies, there was no statistical difference for the variable *a* (t = -0.9866, *P* = 0.3837), *b* (t = 0.0413, *P* = 0.9689), or *k* (t = 1.6928, *P* = 0.1573), indicating no difference in relative humidity output between feeding and non-feeding flies over time. There was strong covariant interaction between CO_2 output over time and the activity of flies (ANCOVA, *P* < 0.0001), with feeding flies producing more CO_2 than non-feeding flies.**

Chapter 4. Concluding Summary

House flies (*Musca domestica* L.) are an annoyance to both humans and animals and are important carriers of disease-causing pathogens. Pesticide-resistant house flies prompted a shift in fly control programs from pesticides to attract-and-kill tactics (Hanley *et al.,* 2009). Currently, the most widely used house fly attractant is (Z)-9-tricosene, the house fly sex pheromone, which serves as an integral attractant in house fly traps and in attract-and-kill control tactics despite its inconsistent attractiveness (Butler *et al.,* 2007; Hanley *et al.,* 2004, 2009). Aside from (Z)-9-tricosene, many malodourous food and oviposition resources of flies emanate semiochemicals that can attract house flies to traps (Geden *et al.,* 2009). However, malodourous attractants are not suitable for deployment in or near humans.

Inoffensive and/or fragrant house fly attractants are needed for use in house fly traps designed for indoor use. To investigate foraging resources for indoor attraction of house flies, I tested the response of flies to various human foods and a floral resource. I also investigated the “fly factor” [food currently or previously fed on by flies attracting more flies than the same type of food kept inaccessible to flies (Barnhart & Chadwick, 1953)] as another potentially effective foraging cue. Because the fly factor is imperceptible to the human nose, it may be suited as a trap bait for indoor attraction and capture of flies.

In my thesis I determined that:

1. House flies are attracted to dandelion honey and dandelion flowers (*Taraxacum officinale* L.).
2. All analytical methods applied to acquire the essential semiochemicals present in dandelion honey or dandelion flowers were not successful.
3. House flies produce and respond to the fly factor.

4. House flies are attracted to both previously fed-on food as well as house fly feces/regurgitate.
5. Feeding and non-feeding house flies do not differ in temperature and relative humidity associated with them, but feeding flies produce more CO_2 than non-feeding flies.
6. Although feeding flies produce more CO_2 than non-feeding flies, elevated levels of CO_2 are not causing the fly factor phenomenon.
7. All methods to acquire the essential semiochemicals causing the fly factor were not successful.

The results presented in my thesis highlight the difficulties in finding novel attractants for house flies, especially for use near humans and indoors. This challenge could be addressed by offering foraging cues that are based on sensory modalities other than, or in addition to, olfaction. Visual foraging cues for house flies have been studied extensively and may be as important as olfactory cues (Conlon & Bell, 1991; Diclaro *et al.,* 2012). Future research should focus on the development of baits that integrate both visual and olfactory cues attractive to house flies.

Regarding the fly factor, it is conceivable that the semiochemicals associated with current or past feeding activities of flies are produced by symbiotic microbes. The proposed concept that feeding and defecating flies inoculate food resources with symbiotic microbes (Hendrichs *et al.,* 1992) is well supported by recent reports that the digestive tract of house flies sustains a diverse microbial community (Gupta *et al.,* 2012). Furthermore, as the sponging mouthparts of flies are capable of taking up only liquid foods or solid foods after being dissolved in watery regurgitates, any microbes – if present in these regurgitates – may become inocula for food resources.

In light of ever increasing evidence for microbial semiochemicals serving as foraging cues for insects (e.g., Becher *et al.*, 2012; Wada-Katsumata *et al.*, 2015), I do anticipate that the hypothesis of a microbe-mediated fly factor will be supported in future studies. The challenge of identifying the microbial semiochemicals that constitute the fly factor, and that attract foraging flies, may be addressed by greatly enhancing semiochemical production. This could be accomplished in follow-up studies by (*i*) allowing flies to feed on, and thus inoculate, diverse types of nutrient media that support

microbial growth, (*ii*) bioassaying the response of flies to media with microbial growth, (*iii*) isolating and mass producing those microbes that attract flies, and (*iv*) accumulating the semiochemicals of attractive microbes for chemical analyses.

4.1. References

Barnhart, C.S. and Chadwick, L.E. (1953) A “fly factor” in attractant studies. *Science*, 117, 104–105.

Becher, P.G., Flick, G., Rozpędowska, E., Schmidt, A., Hagman, A., Lebreton, S., Larsson, M.C., Hansson, B.S., Piškur, J., Witzgall, P. and Bengtsson, M. (2012) Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Functional Ecology*, 26, 822–828.

Butler, S.M., Gerry, A.C. and Mullens, B.A. (2007) House fly (Diptera: Muscidae) activity near baits containing (Z)-9-tricosene and efficacy of commercial toxic fly baits on a southern California dairy. *Journal of Economic Entomology,* 100, 1489-1495.

Conlon, D. and Bell, W.J. (1991) The use of visual information by house flies, *Musca domestica* (Diptera: Muscidae), foraging in resource patches. *Journal of Comparative Physiology*, 168, 365-371.

Diclaro, J.W., Cohnstaedt, L.W., Pereira, R.M., Allan, S.A. and Koehler, P.G. (2012) Behavioural and physiological response of *Musca domestica* to colored visual targets. *Journal of Medical Entomology*, 49, 94-100.

Hanley, M.E., Cruickshanks, K.L., Dunn, D., Stewart-Jones, A. and Goulson, D. (2009) Luring house flies (*Musca domestica*) to traps: Do cuticular hydrocarbons and visual cues increase catch? *Medical and Veterinary Entomology*, 23, 26-33.

Hanley, M.E., Dunn, D.W., Abolins, S.R. and Goulson, D. (2004) Evaluation of (Z)-9-tricosene baited targets for control of the house fly (*Musca domestica*) in outdoor situations. *Journal of Applied Entomology*, 128, 478-482.

Hendrichs, J., Cooley, S.S. and Prokopy, R.J. (1992) Post-feeding bubbling behaviour in fluid-feeding Diptera: concentration of crop contents by oral evaporative excess water. *Physiological Entomology*, 17, 153–161.

Geden, C.J., Szumlas, D.E. and Walker, T.W. (2009) Evaluation of commercial and field-expedient baited traps for house flies, *Musca domestica*, L. (Diptera: Muscidae). *Journal of Vector Ecology*, 34: 99-103.

Gupta, A.K., Nayduch, D., Verma, P., Shah, B., Ghate, H.V., Patole, M.S. and Schouce, Y.S. (2012) Phylogenetic characterization of bacteria in the gut of house flies (*Musca domestica* L.). *FEMS Microbial Ecology*, 79, 581–593.

Wada-katsumata, A., Zurek, L., Nalyanya, G. and Schal, C. (2015) Gut bacteria mediate aggregation in the German cockroach. *PNAS*, 15678–15683.