An Evaluation of Bed Bug (*Cimex lectularius* L.) Host Location and Aggregation Behavior

Matthew Douglas Reis

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Committee Members:

Dini M. Miller

Richard D. Fell

Donald E. Mullins

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ABSTRACT

This study attempts to elucidate bed bug behavior in response to host cues and aggregation cues from conspecifics. Both fed and unfed bed bugs were evaluated to determine differences in behavior with regard to their circadian activities.

Arena bioassays were conducted to determine the bed bug's ability to locate a host from different distances and if the antennae were essential for host location. Starved bed bugs were able to locate a host from 100 cm away. The bed bugs search path became more directed towards the host as the bed bug was placed at closer distances. The bed bugs' mean searching speed was found to be 1.7 cm/s. The bed bugs were able to locate a host even when their antennae were completely removed.

Fed and unfed bed bugs were tested both individually and in groups to determine their attraction towards aggregation cues. Both fed and unfed bed bugs, regardless of sex, were significantly attracted to feces of conspecifics and exuvia of fifth instars.

Finally, bed bugs were observed throughout the night to document their circadian activities after successfully taking a blood meal or failing to take a blood meal. Unfed bed bugs continued to search for a host throughout the night until aggregating together 2 hours prior to photophase. Alternatively, fed bed bugs immediately aggregated together within 30 minutes of a bloodmeal.

Dedication

This labor of love is dedicated to my family.

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Chapter 1. Introduction

The common bed bug, *Cimex lectularius*, is becoming a major pest in human dwellings across the U.S. In the early 1990s, pest management professionals began to receive an increase in complaints regarding bed bug infestations. By 2001, many pest control companies reported 10 and even 100 fold increases in bed bug infestations (Potter 2005). These new infestations were reported in hotels, health care facilities, and used furniture outlets.

The bed bug is a nocturnal blood feeding ectoparasite. The bed bug must obtain multiple blood meals in order to reach adulthood and adults require additional blood meals in order to reproduce. People who are currently living with an infestation typically experience a lack of sleep and a feeling of helplessness as they are bitten numerous times within consecutive nights (Goddard and deShazo 2009).

There is a social stigma associated with bed bugs. Most people assume that those who have poor hygiene and low economic status become infested with bed bugs (Usinger 1966). While it is true that bed bugs thrive in buildings and other structures that are in need of repair, that are crowded with people, or cluttered with furnishings, it is not these factors that cause bed bug infestations. Anyone of any socio-economic status and any human dwelling can become infested.

Bed bug infestations have a number of economic consequences. It is not uncommon for people who have been bitten by bed bugs while staying in a hotel to sue the hotel for thousands of dollars (Harlan et al. 2008). Treating bed bug infested structures is also very costly. For example, the cost of treating a room infested with bed bugs is often in excess of $400. Bed bug treatment often includes one hour of visual inspection, a detailed application of insecticides, and at least some form of customer education regarding how to prevent another infestation (Harlan et al. 2008). Doggett (2008) conservatively estimated that recent bed bug infestations may have cost the country of Australia over $100 million a year.

Since treatment of a bed bug infestation is expensive, many traps and monitors have been designed to minimize the human time and labor involved in controlling bed bug infestations. Bed bug traps and monitors are designed to mimic a host and often contain a heat component, chemical odors, and carbon dioxide (CO_2) (Anderson et al. 2009, Wang et al. 2009). Many traps and monitors are built to exploit host location behavior but research findings on the bed bug's ability to locate a host are conflicting. Rivnay (1932) reported that bed bugs used mainly heat and host odors to locate a host. The study concluded that the bed bug could only detect and orient itself towards the host from a distance of 4 cm and the bed bug's movements were random and non-oriented at greater distances. Alternatively, Marx (1955) reported that bed bugs could locate the host from distances of 150 cm and could locate the host by means other than random searching.

Many recent studies have focused on exploring the bed bug's response to aggregation cues in the hope of developing a monitor that will exploit the bed bug's aggregation behavior. Bed bugs are known to be attracted to bed bug feces, exuvia, and pheromones released from metathoratic glands (Levinson and Bar Ilan 1971, Siljander et al. 2007, Domingue et al. 2010). The blood sucking Hemipteran, *Triatoma infestans*, is also attracted to feces of conspecifics and many chemical extracts of these attractant components of the feces have been applied to *T. infestans* monitors (Guerenstein et al. 1995).

The high cost of treating bed bug infestations, negative public opinion and potential litigation have stimulated greater interest in bed bug research. Recent research has been focused on gathering information about basic host location and aggregation behavior in the hope that this information can be utilized for controlling bed bug infestations. The goals and objectives of this research project were:

1) To evaluate the bed bug's ability to locate a host over different distances and to determine if the bed bug's antennae are essential for location.
2) To determine if feeding status influences the bed bug's response to known aggregation cues (feces, exuvia, or other bed bugs).
3) To evaluate how the bed bug utilizes its time during a nocturnal feeding bout and if feeding success affects its behavior towards aggregating with conspecifics.

Chapter 2. Literature Review

Description of the bed bug

The common bed bug (*Cimex lectularius* L.) is a member of the family Cimicidae within the order Hemiptera. The family Cimicidae is composed of 6 subfamilies containing a combined 23 genera. As of 1981, there were a total of 91 identified species in the family Cimicidae (Ryckman et al. 1981). All Cimicids are haematophagous ectoparasites (Reinhardt and Siva-Jothy 2007). Most species feed primarily on bats and birds, but three species feed on humans: *Cimex lectularius*, the Common Bed Bug; *Cimex hemipterus* Fabricius, the Tropical Bed Bug; and *Leptocimex boueti* Brumpt, a species found in West Africa (Usinger 1966).

The Cimicidae share several distinguishing morphological features. The adult bed bug is 6-7 mm in length, reddish brown and gives off a distinct musty, sweetish odor when disturbed (Harlan 2006).The bodies are broad, oval, and dorso-ventrally flattened. Adults have highly reduced hemelytra that consist only of wing pads. The antennae are always 4 segmented and the labium is 3 segmented (Usinger 1966). Males typically have a pointed abdomen whereas the female’s abdomen is more rounded (Usinger 1966).

Cimicid bugs are unique in their reproductive behavior in that they mate using traumatic insemination (Reinhardt and Siva-Jothy 2007). The insemination process is termed “traumatic” because it involves the male fertilizing the female through an integumental wound. The male’s copulatory organ is called the paramere. When the male mounts the female, he uses his paramere to penetrate through the female’s integument and inject his semen into her abdomen. Females have evolved a specialized paragenital organ to accept the paramere through her body wall called an ectospermalage. The physical morphology of the female paragenital organ and the male paramere are used as taxonomic features to identify Cimicids to the genus level (Usinger 1966).

Like all hemipterans, the bed bug is hemimetabolous (Elzinga 1987). The bed bug's life cycle is composed of 7 life stages: the egg, 5 instars, and the adult. A blood meal is required by each instar in order to develop to the next life stage. Additional blood meals are required by the adults in order to reproduce (Usinger 1966).

Ambient temperature has an influence on the bed bugs' development time. The time required for a bed bug to complete its development from egg to adult ranges from 179.9 days at 18^{o}C to 36.6 days at 33^{o}C. Egg hatch, nymphal development, and adult activity cease at temperatures between 13^{o} and 15^{o}C. Bed bugs can tolerate extremely low temperatures (as low as -15^{o}C) but it is unknown for how long they can survive at these temperatures. Bed bugs are most easily killed by high temperatures, with development terminating between 36^{o} and 37^{o}C (Usinger 1966). Johnson (1941) found that bed bug mortality was 100% when exposed to 43^{o}C for 1 hour.

The relative humidity typically present in human dwellings has a negligible effect on the bed bugs' development rate. Rivnay (1932) found that the development of bed bug nymphs did not change when exposed to relative humidities between 10 to 70%. However, bed bug nymphs frequently died during molting if the relative humidity was below 20%. Female bed bugs can survive in extremely dry conditions (0% RH) for up to 16 days before mortality occurs (Usinger 1966). Extremely low humidity would not typically be encountered in the bed bugs' natural environment such as caves and human dwellings (Benoit et al. 2007).

Bed bug history

Humans are thought to have first come into contact with bed bugs while living in caves in the Middle East (Sailor 1952). Humans undoubtedly used caves as temporary shelter for protection from inclement weather and bed bugs were ectoparasites of bats that also used the caves for shelter. With humans, bats, and cimicid bugs living in close association within caves, the bed bugs could have began to use humans as an alternate host organism (Usinger 1966).

Approximately 8000-5000 B.C., people began to practice agriculture and the practice of agriculture led to the development of civilizations and the construction of villages. As humans left their caves and moved into villages, people would have passively transported bed bugs out of the caves and into their village domiciles. As bed bugs adapted to living with humans and their dwellings, humans would have transported bed bugs whenever and wherever they traveled throughout the world (Usinger 1966).

From the earliest written history, bed bugs have been a pest of humans. Early scholars such as Aristotle (384-322 B.C.), Aristophanes (423 B.C.), and Dioscorides have discussed bed bugs as pests in their writings (Ryckman et al. 1981). The Greek army surgeon Nero (54-68 A.D.) referred to bed bugs as having healing powers that could neutralize snake venom and expel horse leeches. In addition, there is a considerable amount of folklore that surrounds the bed bug as well as names for the bed bug in Indo-European, African, and Asian languages (Usinger 1966). Some common English names for the bed bug include: the red coat, wall flounder, and mahogany-flat (Usinger 1966).

At the beginning of the 20th century bed bugs were common in laundries, factories, upholstery shops, and movie theatres within the U.S. and Europe. Infestations of bed bugs were the worst in poor, overcrowded neighborhoods. However, wealthy homes also had problems with infestations (Potter 2008). In Europe, the growth of cities and the wartime movement increased the spread of bed bugs, aided by the constant movement of people and their infested belongings. At one point, 4 million people in Greater London were infested with bed bugs in their dwellings (Usinger 1966). Hotels and boarding houses in the U.S. were also infested with bed bugs in the early 20th century. Because hotel rooms were known to be infested, travelers would pull beds away from the walls and placed pans of oil under the legs of the beds (Potter 2008). Bed bugs were also found in all major types of transportation, including trains, and moving vans (Whitfield 1939).

At the beginning of the 20th century, control measures to prevent bed bug infestations focused on making habitations less favorable for bed bugs (Potter 2008). Big wooden bed frames were replaced with metal frames that offered fewer places for bed bugs to hide and made bed bug inspections easier (Potter 2008). Clothes from laundries and traveler's suitcases were inspected before they were brought into someone's home (Potter 2008). Also, a variety of chemicals that were applied as both sprays and fumigants were used to control bed bug infestations. Arsenic, mercury, sulfur, cyanide, rotenone, phenol, cresol, naphthalene, Lethane 384, turpentine, gasoline, kerosene, benzene, and alcohol were all applied in various combinations in an attempt to eradicate bed bug infestations. Many of theses chemicals were also very toxic and lethal to humans (Potter 2008).

In 1936, advances in toxicology chemistry introduced Dichloro-diphenyl-trichloroethane (DDT) for the control of insect pests (Ebeling 1975). Initially DDT was used during WWII to control louse-borne typhus and disease-carrying flies, but DDT was also found to be very effective at controlling bed bugs (Potter 2008). By 1950, the liberal use of DDT and changes in cultural practices, such as vigorous housecleaning, had all but eradicated bed bug infestations in the developed world (Busvine 1958). People living in developed countries had enjoyed approximately 50 years of living relatively bed bug free until the recent resurgence in the 1990s (Harlan 2006).

Bed bug resurgence

Determining the time when the current bed bug resurgence began to develop is difficult. Many recent studies suggest that the current spread of bed bugs in the developed world may have begun as early as the 1970's and the 1980's (Hallas et al. 1977). However, most reports suggest that the bed bug resurgence began in the middle to late 1990s. In the United Kingdom, bed bug infestation reports from pest control companies increased from an average of 10 per year in 1997 to nearly 100 in 2000. Similar increases were also reported in Australia. Doggett (2008) surveyed 121 pest managers from across Australia in 2006 and found that bed bug infestations had increased by 4500% between 1999 and 2006. Similarly, in the United States a study by Cornell University found that bed bug infestations have been steadily increasing across the nation since 2004 (Gangloff-Kaufmann et al. 2006).

Arguably the most likely cause of the bed bug resurgence is insecticide resistance (Potter 2008). Bed bugs have been known to rapidly develop resistance to many insecticides. The bed bugs' resistance to DDT was first noted in 1947, only a few years after the introduction of the chemical. More cases of DDT resistance were later confirmed, which resulted in the National Pest Control Association recommending the use of malathion as an alternative to DDT. When bed bugs became resistant to malathion other products were used, such as diazinon, lindane, chlordane, and dichlorvos. During the 1980's, sporadic recurrences with bed bugs were eliminated using organophosphates and carbamates. However, organophosphates and carbamates are no longer registered for indoor use and can no longer be used for bed bug control today.

A chemical, derived from the Chrysanthemum flower, called pyrethrum was found to be highly effective at killing bed bugs. However, pyrethrum in its natural form lacked residual activity (Potter 2008). Today, synthetic varieties of pyrethrum called pyrethroids, are the most commonly used insecticides for treating bed bug infestations. While pyrethroids were originally formulated to increase the residual activity of natural pyrethrum, high levels of resistance to pyrethroid products have already been confirmed (Potter 2008). In addition to pyrethroids, insect growth regulators, such as hydroprene, and products containing chlofenapyr are also being used in integrated management programs to battle modern (post 1990) bed bug infestations.

Medical importance of the bed bug

Due to the resurgence in bed bug infestations around the globe it is important to understand the potential health issues associated with bed bugs (Paul and Bates 2000). The most significant health concern is the allergic response to the bed bug bite. Bed bug bites are typically concentrated on the arms, legs, back, face, and around the eyes (Reinhardt and Siva-Jothy 2007). However, bites may occur wherever the bed bugs can gain access to the skin. The bite of a bed bug and the resulting wheal often resembles the allergic response to many other arthropod or non-arthropod sources (Thomas et al. 2004). Therefore, it is difficult to determine whether or not a wheal on the skin is a bed bug bite.

The bites can also resemble a number of different skin disorders including, pruritic popular, urticarial dermatoses, Grover's disease, acral popular or papullovesicular dermatitis of Gianotti-Crosti, pruritic vesicular disorders, and dermatitis herpetiformis (Tharakaram 1999, Thomas et al. 2004). No one can definitively identify a bed bug bite by visual inspection alone and many bed bug cases have been misdiagnosed. Children have been sent home from school by nurses who diagnosed bed bug bites as chicken pox (Potter 2006). There have been cases where bed bug infestations have been allowed to proliferate due to misdiagnosis of bed bug bites as scabies by medical professionals (Potter 2006). Therefore, it is important that other bed bug evidence be identified before final diagnosis. A detailed history of home environment and work conditions is imperative for diagnosing skin reactions as bed bug bites (Pinto et al. 2007).

One of the reasons bed bug bites are so irritating is because the human epidermal tissue is damaged by the bed bugs piercing-sucking mouthparts. The bed bugs' penetration and probing of the skin tissue is achieved by the use of an extremely flexible fassicle. This fassicle probes throughout the human epidermal tissue in various directions: piercing, cutting, and entering small capillaries and vessels (Usinger 1966). Bed bug oral secretions such as anticoagulants and vasodilators are released into the bite site to inhibit platelet aggregation at the feeding site (Valenzuela et al. 1996, Valenzuela and Ribeiro 1998). It is this combination of fassicle probing and the injection of oral secretions that produce the immune reactions within the bite victim (Usinger 1966).

Reactions to bed bug bites vary within the human population. Some people experience an immediate reaction within the first 48 hours of the bite. Immediate reactions can appear in the form of a wheal, erythema, or vesicle formation. More severe reactions such as bullous reactions and anaphylaxis are rare but have been documented (Parsons 1955, Fletcher et al. 2002). Delayed reactions can also occur. Some reactions may appear up to 9 days after the bite and may look the same as an immediate reaction. However, other people have little or no reaction, even after having been bitten numerous times (Harlan et al. 2008).

We would expect that bed bugs would be ideal vectors of diseases due to the fact they take multiple blood meals and feed on a number of individual hosts during their life cycle. Studies have also found that there are at least 28 naturally occurring pathogens on the bed bugs' body, in their gut, and in their feces. Bed bugs have also been successfully infected with additional pathogens in the laboratory. Human immunodeficiency virus (HIV) was found to be retained in the gut of *Cimex hemipterus* for one week after taking a highly concentrated blood meal. However, HIV was never detected in *C. hemipterus* excreta and the possibility that HIV could be transmitted through a bed bug bite is thought to be remote (Webb et al. 1989). Bed bugs have never been documented to vector any disease even when infected bed bugs were forced to bite uninfected hosts in the laboratory (Ryckman et al. 1981). However, bed bug bites can result in secondary infections in areas where the bites are located. Humans scratching the irritated area of the bed bug bite site can result in secondary infections. Pathology resulting from secondary infections include imptegio, ecthyma, cullulitis, and lymphangitis (Thomas et al. 2004).

Large bed bug infestations can cause the victims to suffer from extreme stress. Many people suffer from nightmares or insomnia due to the fear that bed bugs are feeding on them while they are sleeping. People also develop a feeling of helplessness or lack of control over their lives when they are fed on continuously by the bed bugs. The victims will frequently discard their beds, couches, and any other furniture suspected to be infested with bed bugs. The financial cost of replacing discarded items further adds to the stress of a bed bug infestation (Pinto et al. 2007) .

Bed bug infestations may also create a unique emotional state known as delusory parasitosis. People suffering from delusory parasitosis have often experienced a bed bug infestation and the sufferers are convinced that they are still being bitten, even when no evidence of the infestation remains. Continuously moving from residence to residence can occur as people try to run from the insects. Sufferers of delusory parasitosis may also cause themselves physical harm by placing insecticides on their bodies. People experiencing delusory parasitosis may need to be reassured and convinced that they no longer have bed bugs. However, often the sufferer's infatuation becomes severe and they must be advised to see a physician or psychiatrist (Pinto et al. 2007).

Aggregation behavior in insects

In many species of insects, conspecifics will join together to form aggregations. The most successful insects that aggregate together are the social insects that include bees, ants, and wasps. However, many non-social insects will also form aggregations. Orders of non-social insects that form aggregations include: Blattodea, Diptera, and Hemiptera *(Wertheim et al. 2004).*

The advantages of aggregation behavior include increased access to potential mates, defense from predators, accelerated development, and protection from unfavorable environmental conditions (Dambach and Goehlen 1999). Dambach (1999) found that aggregation density and longevity in German cockroach (*Blattella germanica* L.) nymphs was related to relative humidity. Groups of nymphs would form tight aggregations under conditions of low humidity to protect themselves from desiccation. Looser aggregations would form when the humidity was higher. Moisture evaporation is directly proportional to body surface area, and small animals tend to lose moisture quickly. Therefore, by aggregating together, German cockroach nymphs are able to increase their relative volume, and decrease their surface area, thus decreasing individual moisture loss (Dambach and Goehlen 1999).

Aggregation behavior with conspecifics is also known to accelerate each individual insect's development towards sexual maturity. IzuTsu (1970) found that German cockroach nymphs reared in groups developed faster than German cockroach nymphs reared alone. The inclusion of an individual cockroach into a group of conspecifics was shown to have had an affect on the individual's endocrine system. Specifically, the production of juvenile hormone (JH) was inhibited because of the presence of tactile stimulation received from conspecifics. The decreased levels of JH caused more rapid growth and sexual maturation in the nymphs (IzuTsu et al. 1970).

Often an insect aggregation is located near a favorable resource and as more individuals arrive at the resource, the attractiveness of the location increases. Bartelt (1984) found that sexually mature male fruit flies of *Drosophila virilus* Sturtevant released a pheromone when a food source was found. The male pheromone was attractive to both males and females and as more males arrived at the location, the attractiveness of the aggregation increased. Bartelt (1984) suggested two benefits for fruit flies aggregating at a resource: one was the increased success of finding a mate and the second was the location of a favorable oviposition site by females (Bartelt and Jackson 1984).

The physiological state of an insect affects its response to aggregation stimuli and determines if the individual will or will not join an aggregation. For example, the kissing bug *Triatoma infestans* Klug aggregates around the feces of conspecifics. However, after a blood meal kissing bugs will not aggregate to feces of conspecifics for 24 hours (Schofield and Patterson 1977, Figueiras et al. 1994). It is hypothesized that because feces are also deposited near the host, that kissing bugs could use feces to invert their orientation away from the host (Jander 1963).

Aggregation behavior in Hemiptera

Taxa of Hemiptera that aggregate include: Coreidae; *Notobitus*, Pentatomidae; *Perillus*, Reduviidae; *Triatoma*, and Cimicidae; *Cimex* (Wertheim et al. 2004). Hemipterans form and maintain aggregations using various communication modes that include tactile cues, visual cues, and pheromones. Forming aggregations increases the probability of finding mates, provides opportunistic feeding opportunities, and decreases the probability of being preyed upon.

The bamboo bug, *Notobitus meleagris* Fabricius, forms aggregations to facilitate mating. The male bamboo bug is known to form two types of mating aggregations: harem aggregations and multi-male aggregations (Miyatake 2009). Harem aggregations contain only one male and many females. The dominate male within harem aggregations will defend his females and territory from other males. The location of harem aggregations is often on the most energy rich parts of the plant that are also the most favorable for female oviposition. Multi-male aggregations will form in the absence of a dominate male and these aggregations are composed of both males and females (Miyatake 2009).

The two-spotted stinkbug, *Perillus bioculatus* Fabricius, forms multi-lifestage aggregations. These aggregations contribute to the feeding success of early instars. The two-spotted stinkbug is a gregarious insect that will often feed on Colorado Potato Beetle (CPB) larvae. The early instars of the two-spotted stinkbug are unable to capture large CPB larvae. However, later instars and adult two-spotted stinkbugs have no difficulty capturing large CPB larvae. The early instar two-spotted stink bugs will opportunistically feed on large larvae captured by their older and larger conspecifics. This communal feeding behavior allows the young stinkbugs to conserve energy because the opportunistic feeding decreases their own foraging and handling time of prey (Cloutier 1997).

Rapid dispersal of an aggregation can also be used as a form of defense from predators. An aggregation of southern green stink bugs, *Nezara viridula* L., will quickly disperse when attacked by a predator. When disturbed by a predator, individuals within the aggregation release an alarm pheromone that alerts all members to disperse. Rapid dispersal of the aggregation significantly reduces the risk of predation for any particular individual (Lockwood and Story 1985).

Bed bug aggregation behavior

Bed bugs are nocturnal feeders and will aggregate together in harborages in close proximity to where their host sleeps (Gangloff-Kaufmann et al. 2006). Bed bugs of all developmental lifestages will often aggregate together. Large amounts of bed bug exuvia, feces, and egg casings accumulate in these bed bug aggregations because of the presence of multiple lifestages.

Bed bugs use multiple cues to locate an aggregation (Reinhardt and Siva-Jothy 2007). The cues that influence bed bug aggregation behavior include the tactile stimulation of the antennae, chemical stimuli from the feces of conspecifics, and microclimate factors at the aggregation site that include relative humidity, temperature, and light conditions (Reinhardt and Siva-Jothy 2007).

Levinson (1971) conducted a study to identify bed bug aggregation stimuli and was able to collect what he described as a bed bug body odor. This bed bug body odor was collected by keeping bed bugs in close contact with filter paper shelters. These filter papers impregnated with the body odor were found to be attractive to both male and female bed bugs. Levinson (1971) concluded that the bed bug scent was not used as a sexual attractant but as an aggregation attractant. However, Siljander (2007) repeated Levinson's methods and found that male and female bed bugs were only attracted to filter papers exposed to male bed bugs. Female exposed filter papers were not attractive to bed bugs of any developmental lifestage. Siljander (2007) also found that early instars also left odors on filter paper and these scented filter papers were attractive to other early instars.

Siljander (2008) was able to collect a bed bug body scent that he termed an "airborne aggregation pheromone". The aggregation pheromone was collected from the headspace above bed bugs contained in jars. The collected volatiles contained many chemical components including, (E)-2-hexenal, (E)-2-octenal, (2E, 4E)-octadienal, benzaldehyde, nonanal, decanal, sulcatone, (+)-limonene, (-)-limonene, and benzyl alcohol. Adult males, adult females, and nymphs were all found to be attracted to the "airborne aggregation pheromone" in dual choice chamber olfactometer tests.

Interestingly, Siljander (2008) concluded that the airborne aggregation pheromone was only a bed bug attractant but not an arrestant.

Bed bugs have also been found to be attracted to dichloromethane soluble elements of 5th instar exuvia. However, only male bed bugs are attracted to these extracts (Domingue et al. 2010). The 5th instar exuvia do not possess metathoratic glands such as those found in the adults; however, 5th instars do possess glands on the first three segments of the dorsal region of their abdomens. The dorsal glands of the 5th instar nymphs release many of the same molecules that are found to be released from the metathoratic glands of adults (Feldlaufer et al. 2010). The male bed bugs may be attracted to 5th instar exuvia because exuvia may indicate the presence of newly emerged virgin females (Domingue et al. 2010).

Olson (2009) found that bed bugs of all developmental stages were attracted to the feces of conspecifics. Olson (2009) also found that bed bugs became increasing less attracted to bed bug feces the longer they went without feeding. Bioassays where the bed bugs were starved for periods greater than 14 days indicated that bed bugs did not aggregate in harborages stained with bed bug feces. It was hypothesized that alternative behaviors such as host-seeking took precedence over aggregation behavior in these severely starved bed bugs. When the bed bugs antennae were removed the bed bugs could not locate the fecal stained filter paper.

Levinson (1973) was able to locate numerous bristles on the bed bugs' antennae that served a tactile function and induced aggregation behavior. The bed bugs' remained immobile when its antennae and body were in contact with a surface or with other bed bugs, a behavior defined as thigmotaxis (Johnson 1941). Johnson (1941) observed a bed bug is this state of immobility for up to 7 weeks. In addition, a separate study by Olson (2009) found that receptors used to locate the feces of conspecifics were also located on the antennae.

Bed bugs aggregated together are able to slow dehydration and prevent desiccation. The blood meal is the bed bugs' only source of moisture and they may have to survive months between blood meals. An early instar can reduce its moisture loss in half by aggregating with 20 other early instars (Benoit et al. 2007). In addition, Benoit (2007) found that bed bugs will spend equal amounts of time in the middle and at the edges of an aggregation, indicating that all individuals benefit equally from the moisture saving function of an aggregation.

Bed bugs cannot aggregate all the time and there are several factors that will cause a bed bug to leave an aggregation, such as feeding. However, another reason to leave the aggregation is to avoid mating. Adult female bed bugs will disperse if mixed-sex aggregations become composed of 20 individuals or greater. Pfiester (2009) suggested that females would leave an aggregation to escape the mating attempts of males. Pfiester (2009) found that females left an aggregation with a 1:1 sex ratio as the population increased from 10 individuals to 20 individuals. However, females would not disperse from an aggregation if it was made entirely of females, even if the number of individuals within the aggregation increased. Interestingly, the male bed bugs would begin to leave an aggregation as the proportion of females in the aggregation decreased. Male bed bug dispersal is thought to be due to a need to find mates (Pfiester et al. 2009).

Bed bugs will also disperse from an aggregation if disturbed. The bed bug possesses a scent gland between its mesothorax and metathorax from which an alarm pheromone is secreted that causes other bed bugs to become irritated. The irritation causes bed bugs to scatter and leave the aggregation. The alarm pheromone is composed of two molecules that are also found in the aggregation pheromone: (E)-2-hexenal, (E)-2-octenal (Levinson and Bar Ilan 1971). However, when large amounts of these molecules are discharged, they stimulate an alarm response (Siljander et al. 2008). The bed bug is not the only Hemipteran that possesses a multifunctional pheromone that is attractive at low concentrations and repellent at high concentrations. The Hemipteran, *Nezera viridula,* has also been found to possess a multifunctional pheromone (Lockwood and Story 1985).

One disadvantage of congregating in a large aggregation of bed bugs is the possibility of individuals becoming infected with fungal diseases. *Aspergillus flavus* has been known to infect laboratory colonies of the bat bug, *Paracimex* (Usinger 1966). Fungal growth can also infect and cause death in laboratory colonies of bed bugs (Kemper 1936). However, it has not been determined if large field populations of bed bugs have ever become infected with a fungal pathogen (Usinger 1966).

Host location behavior

It is believed that haematophagy in insects arose independently on several occasions during the Jurassic and Cretaceous period. Evolution of the blood sucking behavior in each occasion occurred along one of two main routes: prolonged close association with vertebrates; or morphological pre-adaptation for piercing mouthparts (Waage 1979). It is estimated that there are approximately 14,000 species of insects from 5 orders that feed on the blood of vertebrates. However, only 300-400 species are of medical and veterinary importance (Lehane 2005).

Insects that feed on the blood of other species must be able to locate their hosts. Lehane (2005) explained insect host location behavior in three phases: the appetitive searching phase, the activation and orientation phase, and the attraction phase. Appetitive searching is brought on by hunger and causes the insect to leave its resting place and move in a non-oriented manner. When a host sensory cue is detected the insect switches to an oriented host location behavior. This oriented location behavior is the activation and orientation phase. The final phase is the attraction phase. The attraction phase occurs when host sensory cues bring the insect into the immediate vicinity of the host and the insect makes contact.

The blood feeding insect will exploit a variety of host sensory cues to locate a host. These sensory cues can include CO_2, other olfactory cues, radiant heat, visual cues and tactile cues. The way an insect exploits each of these sensory cues is very specific. For example, CO_2 acts as an alerting stimulus in the kissing bug, *Triatoma infestans*. When CO_2 is detected, the kissing bug will begin a non-oriented searching behavior. When the kissing bug arrives in close proximity of its host, radiant heat elicits an orientation response. However, actual host recognition relies on contact receptors located within the kissing bug's stylet food canal. If the kissing bug detects high energy nucleotides, such as ATP, then the bug will begin feeding (Schofield 1994).

A blood sucking insect's period of activity usually occurs at a specific time (often at night) within a 24 hour period. The bed bug and the kissing bug will typically hide during the day and feed at night when their host is asleep. However, these periods of activity can change or intensify with changes in the insect's physiological state. For example, an insect's period of appetitive searching activity can increase as hunger increases. If the kissing bug is severely starved, it will feed during the day when its host is normally awake even though there is a greater risk of being detected (Lehane 2005).

Host location behavior in bed bugs

The bed bug's foraging activity occurs at certain times within a 24 hour period. Mellanby (1939) conducted a study of a natural bed bug infestation in London and found that bed bugs were most active between the hours of 3 a.m. and 6 a.m. The bed bugs' rhythm of activity remained consistent even when the room containing the infestation was kept in complete darkness for 2 days. However, the bed bugs' activity was slightly inhibited when artificial lighting was used during a 2 day period (Mellanby 1939).

The bed bug uses a combination of host sensory cues to locate a host. Bed bugs are attracted to human breath, CO_2, other chemical odors, and heat (Usinger 1966). However, the bed bugs' sensitivity to each of these sensory cues is variable. Rivnay (1931) found radiant heat to be the most important factor in stimulating bed bugs to obtain a blood meal. However, Rivnay (1932) also found that bed bugs cannot detect the heat of a host at distances greater than 4 cm, and that human odors were attractive to bed bugs. These odors included sebum and cerumen, which are secretions from human glands in the skin and ear. However, these odors were also only detectable by the bed bug over short distances (less than 4 cm). In contrast, the bed bug has been shown to detect CO_2 over greater distances; Anderson (2009) for example was able to attract bed bugs to a CO_2 source from a distance of 86 cm.

Traps and monitors have been developed for the control of bed bug infestations. The traps often contain a heat component, a CO_2 component and a chemical odor component. These traps are intended to mimic a host and the objective of the trap is to capture hungry bed bugs that are foraging for a blood meal. Bed bug populations can be reduced using a monitor that mimics a host; however, the population cannot be completely eradicated (Anderson 2009). Anderson (2009) tested various traps that used heat, CO_2, and chemical odors as bed bug attractants. Although all traps were able to catch bed bugs, traps baited with only CO_2 captured significantly more bed bugs than traps baited with heat and host odors. In a separate study, Wang (2009) was able to demonstrate that traps baited with CO_2 were able to capture large numbers of bed bugs, including many bed bugs that were not observed during visual inspection of infested rooms.

Recent research has been focused on the chemical ecology of aggregation behavior and host location behavior of the bed bug. The information gathered from host location research has already been implemented into monitoring traps used for commercial use. The goal of the following research was to continue to evaluate the basic aggregation and host location behavior of the bed bug with the hope that new information regarding bed bug behavior can be used in the future to improve traps and monitors used for bed bug control.

Chapter 3. Host searching in the bed bug, *Cimex lectularius* (L.): an analysis of movement behavior.

Introduction

A resurgence of infestations of the bed bug, *Cimex lectularius* L., has been reported in many areas of North America, Europe, and Australia (Boase 2001, Hwang et al. 2005, Potter 2005, Harlan 2006). These reports of infestations have been documented in apartment complexes, homeless shelters, hotels, and single family residences (Harlan et al. 2008). The propagation of bed bugs may be attributed to increased international travel, limited availability of effective insecticides, and insecticide resistance (Romero et al. 2007). The increase in bed bug infestations is a cause for concern because of the severity of the bed bug's bite and the allergic reactions the bites can cause (Venkatachalam and Bhavani 1962). A wheal will often form at the site of the bite, along with inflammation and irritation. The bed bug is not known to vector any diseases to humans. However, there is the potential for secondary infections caused by the victim scratching the irritated site caused by the bite (Goddard 2009).

An insect's movements are involved in a large spectrum of fundamental processes (Benhamou 2004). For example, the bed bug must be able to navigate within its environment, disperse from less favorable conditions to more favorable conditions, and food search which involves finding a host to obtain a blood meal. Bed bugs will actively move and search for a host to obtain a bloodmeal. The more efficient a bed bug is at locating the host, the more its path should be close to a straight line linking the starting point to the goal or host (Benhamou 2004).

Many studies on the ability of blood sucking insects to locate a host from a distance have been conducted on flying insects (Bar-Zeev et al. 1977, Bursell 1984b, a, Sutcliffe 1986). Many flying insects use odor plumes to locate a host from a distance. Host cues include carbon dioxide (CO_2) and lactic acid (Geier and Boeckh 1999). However, when these olfactory stimuli are released individually in bioassays at natural dose rates they only attract about 50 % of the insects a natural host would attract (Hargrove et al. 1995). These results suggest that a combination of host cues is used by the insect to locate a host.

The receptors that detect host cues are primarily located on the antennae of blood feeding insects. These receptor sensilla include mechanoreceptors, thermoreceptors, and chemoreceptors. The stimulation of these peripheral receptors is ultimately processed by the insect's central nervous system and the insect is able to locate the host (Davis and Sokolove 1976).

In blood feeding insects, the success of locating their next blood meal depends on the insect's closeness of association with the host. Permanent ectoparasites are always in close contact with the host and only by accident will find themselves more than a few millimeters from the host. On the other end of the spectrum are the temporary ectoparasites such as blood feeding flies that do not remain in close vicinity to the host (Chapman 1982). The bed bug is a temporary blood feeding ectoparasite and harborages in close proximity to where the host rests. When the bed bug becomes hungry it will actively search for a host to obtain a blood meal, which it must obtain in order to mature to the next life-stage. In addition, female bed bugs require additional blood meals in order to produce eggs (Johnson 1941).

A series of behaviors occur when a hungry insect detects the presence of a host. The host cues may change the behavioral awareness of the insect without causing any observable insect activity. However, as subsequent host cues are detected by the insect, an orienting behavior is typically exhibited. A blood feeding insect will continue to move in an oriented behavior as more information contained in the host cues is detected and processed (Lehane 2005).

Triatoma infestans Klug is able to fly, however it walks to locate a host because it lives in close association with its human host. *T. infestans* will orient itself towards sources emitting CO_2 or lactic acid (Barrozo and Lazzari 2006). Not only does *T. infestans* orient itself towards a host because of the olfactory stimulation of CO_2, but also by the mechanical stimuli of the host's breathe over *T. infestans'* body. Once *T. infestans* is in the vicinity of the host, heat and warmth from the host will orient *T. infestans* to the host's exact location and feeding will commence (Schofield 1994). Because *T. infestans* and the bed bug take harborage in close proximity where the host rests, it is probable that the bed bug's searching behavior follows a similar sequence as *T. infestans*.

Research findings on the bed bugs' ability to locate a host are conflicting. The bed bug is attracted to host cues such as heat, CO_2, and other host odors. Rivnay (1932), using himself as the host, found that bed bugs would search at random until within 4 cm of the host, at which point the bed bug's movement became directly oriented towards the host with the its proboscis extended. However, Marx (1955) reported that bed bugs were able to locate a host by means other than chance. Marx (1955) concluded that the bed bug was able to perceive a host from a distance of 150 cm.

The purpose of this study was to investigate the bed bug’s host location behavior. The following experiments were carried out to evaluate the path of bed bug’s movement in relation to its distance from the host. Potential differences in the host location behavior between males and females were also investigated. The bed bug’s antennae were also completely ablated to determine if the antennae were essential for host location.

Materials and Methods

Origin and maintenance of *Cimex lectularius* colony. Bed bugs were collected from a human dwelling in Cincinnati, Ohio and were kept in plastic jars (7cm x 5 cm) containing a cardboard substrate that provided a surface on which they could walk and oviposit. Bed bugs were maintained in an environmental chamber at 23-26^{o}C, 40-70% r.h., and a L12:D12 photoperiod. Bed bugs were fed once a week using an artificial membrane system or on a human volunteer. The membrane system used chicken blood treated with sodium citrate as an anticoagulant.

Preparation of bed bugs. Each individual bed bug was fed and placed on a 2cm^{2} filter paper shelter folded at approximately 45^{o}. This bed bug was then left to starve within its respective shelter for 2-3 weeks within an environmental chamber under 23-26^{o}C, 40-70% r.h., and a L12:D12 photoperiod. Prior to the experiment, the bed bug and its shelter was removed from the environmental chamber and placed inside the room containing the host location test arena to acclimate for 2 hours.

Antennectomized bed bugs. All four of the bed bugs antennal segments (scape, pedicel, and both flagella segments) (Figure 1) were removed (Figure 2) to determine if these sensory structures were essential for host location. An individual bed bug was chilled on ice for 2 minutes and removal of antennae was performed using a scalpel 24 hrs before the bioassay. Control insects were chilled and handled similarly to test insects.

Bioassay arena. The test arena (Figure 3) used was a 2.44 m x 1.22 m galvanized steel box. The walls of the arena extended to a height of 20 cm and the floor was covered with brown kraft paper (40#, Westrick Paper Company, Jacksonville, FL). A large sheet of transparent plexiglass (0.10 inch, Piedmont Plastics, Charlotte, NC) was used to cover the box during the bioassays. The room was illuminated using two red filtered lights to minimize visual stimuli and the environmental conditions within the room were maintained at 22-25°C, 20-40% r.h. and a 12L:12D photoperiod.

Bioassay protocol. An individual bed bug within a shelter was placed at a distance of 25, 50, or 100 cm from the host. The researcher would position himself at one end of the test arena and rest his forearm within the arena. In addition, the researcher's breath was directed into the bioassay arena towards the researcher's forearm located within the bioassay arena. As the bed bug left its shelter to locate the host, the bed bug's movement within the arena was traced onto the plexiglass frame using a water-based paint marker (Poster-Paint, Sharpie®, Oakbrook, IL). A stop watch was used to measure the time it took for the bed bug to locate the host. After each replication, the plexiglass lid with the tracing of the bed bug's movement was photographed using a digital camera (Samsung TL34HD, Samsung®, USA). These images (Figure 4, 5, and 6) of the bed bug's movement were then uploaded onto a computer and the total distance traveled by the bed bug was measured using ImageJ software (Image J, Image Analysis and Processing in Java, National Institute of Health, Bethesda, MD). Two insects were bioassayed individually each night. After four replications, the paper covering the test arena floor was replaced.

A number of variables were recorded during each host location bioassay. The search time was a measurement of the bed bug's initial departure from the shelter to the point of locating the host. The search time was measured in seconds using a stopwatch (Model SF002, Innovative Time Corp., Carlsbad, CA). A searching speed could then be calculated by dividing the total distance traveled by the search time. The final variable recorded was the straightness of the bed bug's path. The straightness of path was derived by an equation used by Heisswolf (2007). Straightness, S=L/D, was calculated as the quotient of distance from the shelter to the host (L) divided by the total distance traveled by the bed bug (D). The straightness value could range from 0.0 (for a highly convoluted path) to 1.0 (linear).

Statistical analysis. The mean search time, distance traveled, search speed, and straightness of path for bed bugs at different distances were analyzed using Analysis of Variance (ANOVA). In addition, all variables were compared using Tukey-Kramer HSD to determine if the means were significantly different ($P \leq 0.05$). The Student's *t*-test was used to compare host location variables between bed bugs with antennae intact and antennectomized bed bugs. Values of $P \leq 0.05$ were used to indicate statistical significance. All statistical procedures were calculated with the software package JMP®8 (JMP8 2008).

Results

Mean bed bug search time. Mean search time for male and female bed bugs is summarized in Table 3.1. There was a significant interaction between the distance the bed bug was located from the host and the sex of the searching bed bug (d.f. = 5, $F = 3.9$, $P \leq 0.0268$). The female bed bug's search time at 25 cm from the host was not significantly different than the search time from 50 cm. However, the female bed bug was able to locate a host in significantly less time at 25 cm or 50 cm from the host than when the host was 100 cm away.

Similar to female bed bugs, the male bed bug's search time when the host was 25 cm away was not significantly different than the search time when the host was 50 cm away. The male bed bug was able to locate a host in significantly less time at a distance of 25 cm or 50 cm than at a distance of 100 cm away.

The male and female bed bugs' mean search time at 25 cm and 50 cm was not significantly different. However, female bed bugs were able to locate a host at 100 cm in significantly less time than males.

Mean distance traveled from shelter to host. The mean distance traveled by the bed bug to find the host is summarized in Table 3.1. There was a significant interaction between the distance the bed bug was located from the host and the sex of the searching bed bug (d.f. = 5, $F = 3.4$, $P \leq 0.0437$). The distance traveled by female bed bugs located 25 or 50 cm from the host was significantly less than that traveled when the host was located 100 cm away. However, the female bed bugs' distance traveled from 25 cm was not significantly different than the distance traveled at 50 cm from the host.

Similar to female bed bugs, the male bed bugs' distance traveled to the host was significantly less when the host was 25 cm or 50 cm away than when the host was 100 cm away. However, the distance traveled by the male bed bug when the host was 25 cm away was not significantly different than the distance traveled by the bed bug when it was 50 cm away from the host.

The female and male bed bugs' mean distance traveled to the host was not significantly different from a distance of 25 cm or 50 cm. However, females traveled a significantly shorter distance than males when locating a host at 100 cm away.

Mean speed traveled by a searching bed bug. The mean speed traveled by the bed bug to find the host is summarized in Table 3.1. There was no significant interaction between the bed bugs distance from the host and the bed bug's sex (d.f. = 5, $F = 1.3$, $P =$

0.2908). The walking speed of a bed bug searching for a host ranged between 1.4 ± 0.2 cm/s and 1.9 ± 0.2 cm/s.

Straightness of the bed bugs' path towards a host. The mean straightness of the bed bugs' path to find the host is summarized in Table 3.1. There was a significant interaction between the distance the bed bug was located from the host and the sex of the searching bed bug (d.f. = 5, $F = 0.4$, $P \leq 0.6745$).The female bed bugs' path to the host was significantly more linear and less convoluted at a distance of 25 cm than from 50 cm and 100 cm. The straightness of the female bed bugs' path when the bed bug was 50 cm away from the host was not significantly different than when the bed bug was100 cm from the host.

The male bed bugs' path towards the host was significantly more linear and less convoluted when the host was at a distance of 25 cm away than when the host was 50 cm and 100 cm away. The male bed bugs' path straightness at 50 cm was not significantly different than the male bed bugs' path at 100 cm.

The straightness of the female bed bug's path was not significantly different than male bed bugs' path from 25, 50, or 100cm

Antennectomized bed bug searching behavior. The mean search time, distance traveled, mean speed, and mean straightness of antennectomized bed bugs are summarized in Table 3.2. The absence of antennae significantly affected the bed bugs' mean time to locate a host, mean searching speed, and the mean straightness of its path. The mean search time of a bed bug without antennae at 25 cm from the host was significantly greater than that of a bed bug with antennae (d.f.=1, $F = 5$, P=0.0364). The mean searching speed of a foraging bed bug with its antennae removed was significantly slower than the searching speed of a bed bug with antennae (d.f.=1, $F = 3$, P=0.0812). The bed bugs' path straightness was significantly less linear and more convoluted when its antennae were removed compared to a bed bug with its antennae intact (d.f.=1, $F = 8$, P=0.0101). However, the mean distance traveled by a bed bug without antennae at 25 cm from the host was not significantly greater than the mean distance traveled by a bed bug with antennae at 25 cm from the host (d.f.=1, $F = 3$, $P \leq 0.0812$).

Discussion

Once the bed bugs detected the host's presence, they were observed to move out and around the paper shelter. Often, the bed bug would leave the shelter for a short distance only to immediately return back to the harborage. These very short initial excursions could possibly have been preliminary localized searches used to gather more host location or harborage orientation information. However, the bed bug would only perform 1-2 of these small localized searches before it left the harborage for good. Once the bed bug entered a close distance from the host, 4 cm and less, its antennae and proboscis were outstretched and pointed toward the host. A similar observation was made by Usinger (1966).

Although the bed bug's preference for individual host sensory cues was not tested, it appeared as if warm breath was the most attractive cue. Carbon dioxide is present in human breath and bed bugs have been reported to be highly attracted to CO_2 (Wang et al. 2009). Warm breath also appeared to be an important main host cue that caused the bed bug to abort its state of rest upon the paper shelter. In addition, the bed bug was often observed to move towards the source of the researcher's breath. This is contradictory to observations made by Kemper (1936) who reported the bed bug's aversion of “drafts” of air because its environment often involves microhabitats with little to no air movement. Barrozo (2006) reported that *Rhodnius prolixus* Stal and *T. infestans*, two blood feeding Hemipterans, could locate a host by following pulses (similar to the rhythmic breathing of a host) of CO_2.

The bed bug was observed to locate a host at closer distances better than at farther distances which could be explained by the number of antennal receptors located on the bed bug’s antennae. The bed bug has 56 antennal receptors. The majority of these receptors are located on the distal part of the terminal segment and serve a probable olfactory function (Levinson et al. 1974, Lehane 2005). In comparison, *T. infestans* has over 2900 antennal receptors which enable *T. infestans* to adopt both a peridomestic and silvatic lifestyle (Lehane 2005). However, the bed bugs low number of antennal receptors requires the bed bug to remain in close proximity where the host sleeps such as mattresses, bed frames, and headboards.

Male and female bed bugs exhibited no significant difference in their ability to locate a host except that female bed bugs were able to detect a host more efficiently at 100 cm than male bed bugs. The female’s ability to locate a host more efficiently than males from 100 cm cannot be explained by the number of antennal receptors. Although Singh (1996) reported that male tropical bed bugs, *Cimex hemipterus* Fabricius, possessed more antennal receptors than female tropical bed bugs, a similar study by Levinson (1974) found that male and female bed bugs, *C. lectularius,* exhibited no sexual dimorphism of antennae receptors. Alternatively, the female bed bug possessed more olfactory receptors on its rostrum than male bed bugs. It is perhaps possible that the additional sensilla on the rostrum of the female bed bug, *C. lectularius*, enabled it to locate a host from 100 cm more efficiently than males.

The females greater energy demands required to produce eggs could explain their ability to locate a host from 100 cm better than males. Hunger has been shown to heighten the responsiveness of blood feeding insects to host cues and increase the overall activity of the insect (Brady 1972). Female tropical bed bugs, *C. hemipterus*, are documented to take larger blood meals than tropical bed bug males (Yanovski and Ogston 1982). Yanovski (1982) explained that larger bloodmeal intake by females could be explained by the greater metabolic demand placed on the female by the need to produce eggs compared to the smaller cost to the male in producing sperm. Hence, a female starved for 14 days might have a greater stimulus to search for a host and feed due to her comparative higher level of starvation in comparison to a male that is also starved 14 days.

The bed bugs ability to locate a host was significantly decreased when its antennae were removed. The bed bug was observed to travel greater distances, its path was more convoluted, and the bed bug walked slower. Wigglesworth (1934) observed that the blood sucking Hemipteran, *R. prolixus,* would also walk slower and exhibit a slower response in recognizing a host when its antennae were removed. Although the bed bug's searching ability decreased with the removal of its antennae, the bed bug was still able to detect and locate the host. This finding indicates that the bed bug possesses host detection receptors on other parts of its body. Singh (1996) was able to identify both mechanoreceptors and gustatory receptors on the rostrum of *C. hemipterus*. In addition, Singh (1996) also identified mechanoreceptors and gustatory receptors on the front, middle, and hind tibia of *C. hemipterus*. Chemoreceptors have been identified on the maxillary palps of *Aedes aeqypti* L. that respond to small increments of CO_2 and a single pulse of CO_2 that is greater than background concentrations (Grant et al. 1995).

The bed bug, with or without antennae, may be able to sense substrate vibrations given off by the host. The bed bug is thigmotactic and its body was in close contact with the shelter which was placed inside the galvanized steel bioassay arena. Although the bed bug may not be able to extract directional information from substrate vibrations, it is possible that initial vibration disturbance could alert the bed bug to the presence of a potential host. Combinations of human breath and vibration has been shown to increase the movement of the flea, *Ctenocephalides canis* Latreille (Sasa 1957).

The results from our experiment could improve the use and placement of bed bug traps that mimic a host. Wang (2009) observed that hungry bed bugs from infested dwellings will often travel through hallways and into adjacent rooms. Wang (2009) suggested that monitors containing heat, CO_2, and other chemical odors should be placed in unoccupied infested rooms to prevent the hungry bed bugs from dispersing to adjacent rooms. Our results suggest that these monitors should also be placed in close proximity to harborages in areas where the bed bug can most efficiently locate the trap. Placing traps that use host cues as attractants close to or on the area where the host rests may keep hungry bed bugs in a localized area and reduce the population searching in other locations, preventing dispersion.

Our results indicate that bed bugs are more efficient at locating a host from relatively close distances in less time and movement than from farther distances. The removal of the bed bugs' antennae reduced their ability to locate a host from a close distance.

However, the bed bug was still able to detect the presence of a host when its antennae were removed, suggesting that the bed bug possesses sensory structures located on additional areas of its body. More research on host searching behavior is required to determine how bed bugs locate a host from distances greater than 100 cm and to determine on which parts of the body these additional host-sensory structures are located.

Table 3-1. Absolute movement variables of male and female bed bugs when a host is present at 25, 50, and 100cm.

Distance (cm)	Sex	Replications	Mean Time (seconds) and SEM	Mean Distance Traveled (cm) and SEM	Mean Speed (cm/s) and SEM	Mean Straightness (distance/distance traveled) and SEM
25	**Female**	**10**	26 ± 3 **A**	36.3 ± 2.1 **A**	1.5 ± 0.2 **A**	0.71 ± 0.04 **A**
	Male	**10**	23 ± 3 **A**	42.1 ± 5.0 **A**	1.9 ± 0.2 **A**	0.65 ± 0.06 **A**
50	**Female**	**8**	92 ± 7 **A**	127.4 ± 17.4 **A**	1.4 ± 0.2 **A**	0.43 ± 0.04 **B**
	Male	**8**	89 ± 8 **A**	125.1 ± 15.1 **A**	1.6 ± 0.3 **A**	0.44 ± 0.05 **B**
100	**Female**	**8**	193 ± 27 **B**	332.4 ± 30.7 **B**	1.9 ± 0.2 **A**	0.32 ± 0.03 **B**
	Male	**8**	296 ± 49 **C**	468.4 ± 67.6 **C**	1.6 ± 0.1 **A**	0.25 ± 0.04 **B**
	F		24	35	1	17
	P		0.0001	0.0001	0.409	0.0001

Shown are the means (±SEM) of time required to locate host (seconds), distance traveled to locate host (centimeters), traveling speed of bed bug searching for a host (centimeters/second), and the straightness of the bed bugs' path to locate host (distance of host from bed bug/distance traveled by bed bug to locate host). P denotes the significance of the Analysis of Variance (ANOVA); df = 5. Values followed by same letter within columns indicate no statistical difference based on the Tukey HSD analysis, α = 0.05.

Table 3-2. Absolute movement variables of adult bed bugs when antennae are intact or fully removed when a host is present at 25 cm.

Antennae	Replications	Mean Time (seconds) and ± SEM	Mean Distance Traveled (cm) and ±SEM	Mean Speed (cm/s) and ± SEM	Mean Straightness (distance/ distance traveled) and ± SEM
Intact	**10**	28 ± 3	39.5 ± 3.3	1.5 ± 0.2	0.66 ± 0.07
Absent	**20**	126 ± 31	129.7 ± 34.9	1.1 ± 0.1	0.42 ± 0.06
F		5	3	6	8
P		0.0364	0.0812	0.0190	0.0101

Shown are the means and (±SEM) of time required to locate host (seconds), distance traveled to locate host (centimeters), traveling speed of bed bug searching for a host (centimeters/second), and the straightness of the bed bugs' path to locate host (distance of host from bed bug/distance traveled by bed bug to locate host). P denotes the significance of the Student's *t*-test, $\alpha = 0.05$.

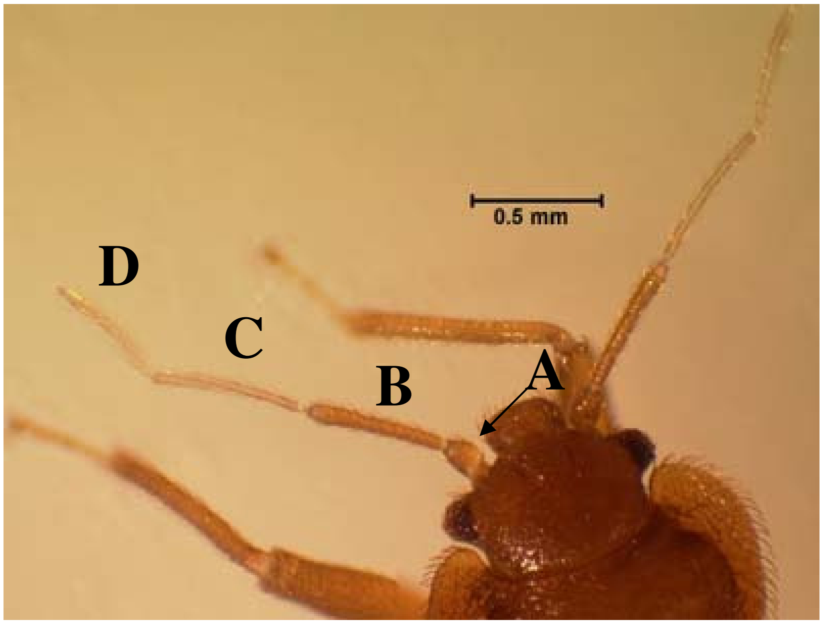

Figure 3-1. Bed bug, *Cimex lectularius* L., antennae: A) scape, B) pedicel, C) flagellum segment, D) distal flagellum segment.

Figure 3-2. Antennectomized bed bug, *Cimex lectularius* L. All antennal segments removed.

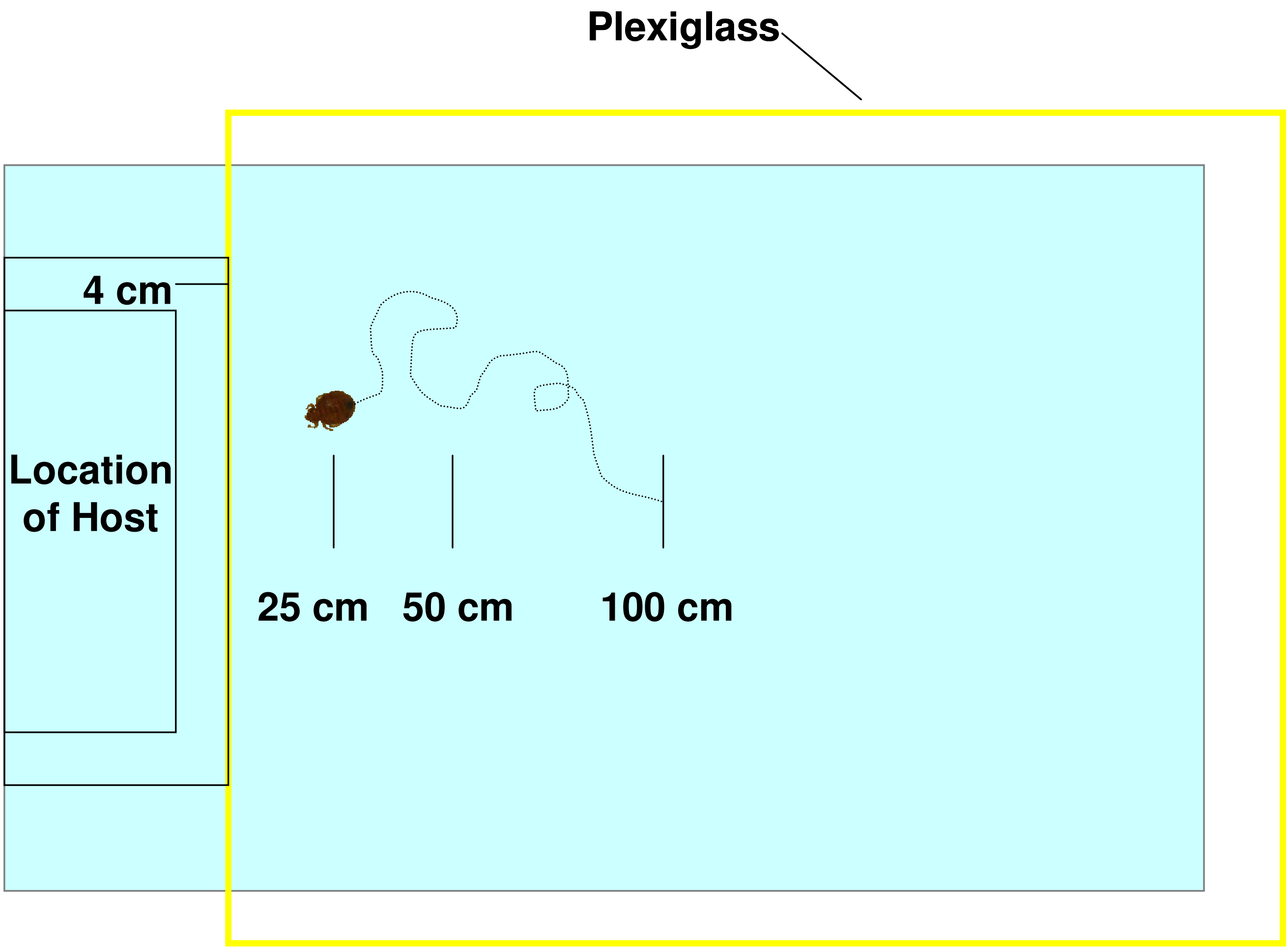

Figure 3-3. Diagram of bioassay arena used to test the bed bug's host location behavior. The researcher positioned his forearm and directed his breath into the bioassay arena. The bed bug was placed at 25, 50, or 100 cm away from the host.

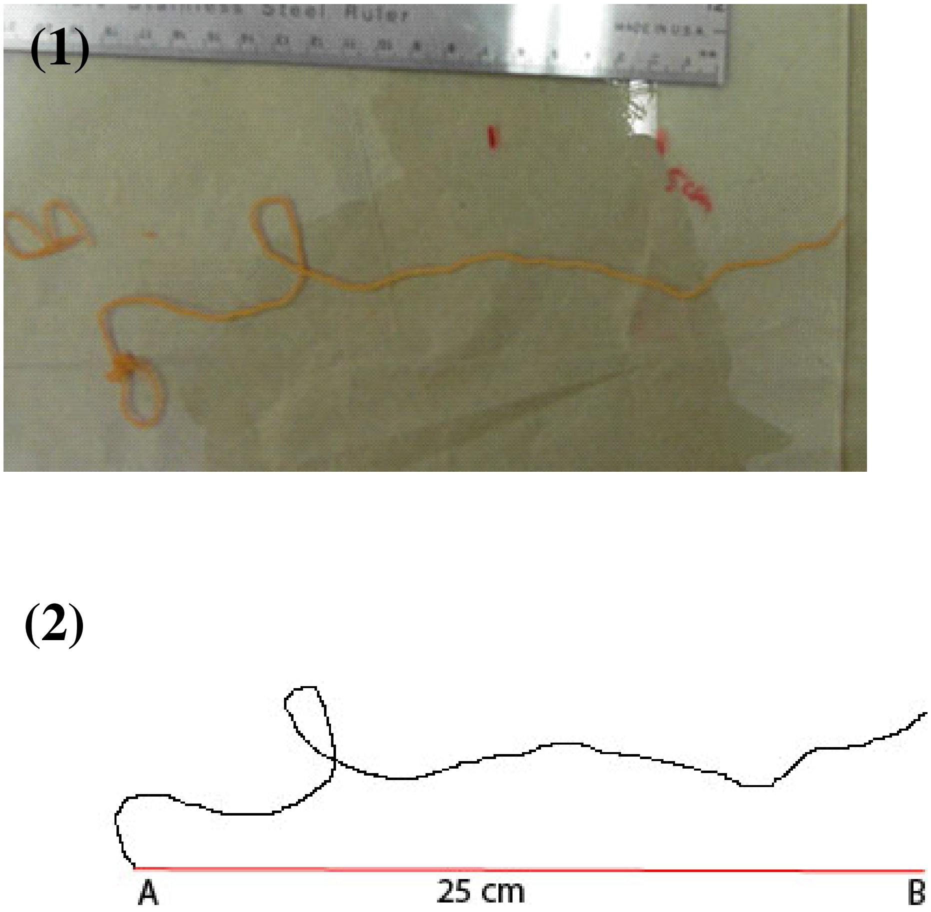

Figure 3-4. (1) Digital photograph taken of a plexiglass sheet showing researcher's trace of the bed bug movement from 25 cm from the host. (2) Software conversion of bed bug's movement path from shelter (A), to host (B).

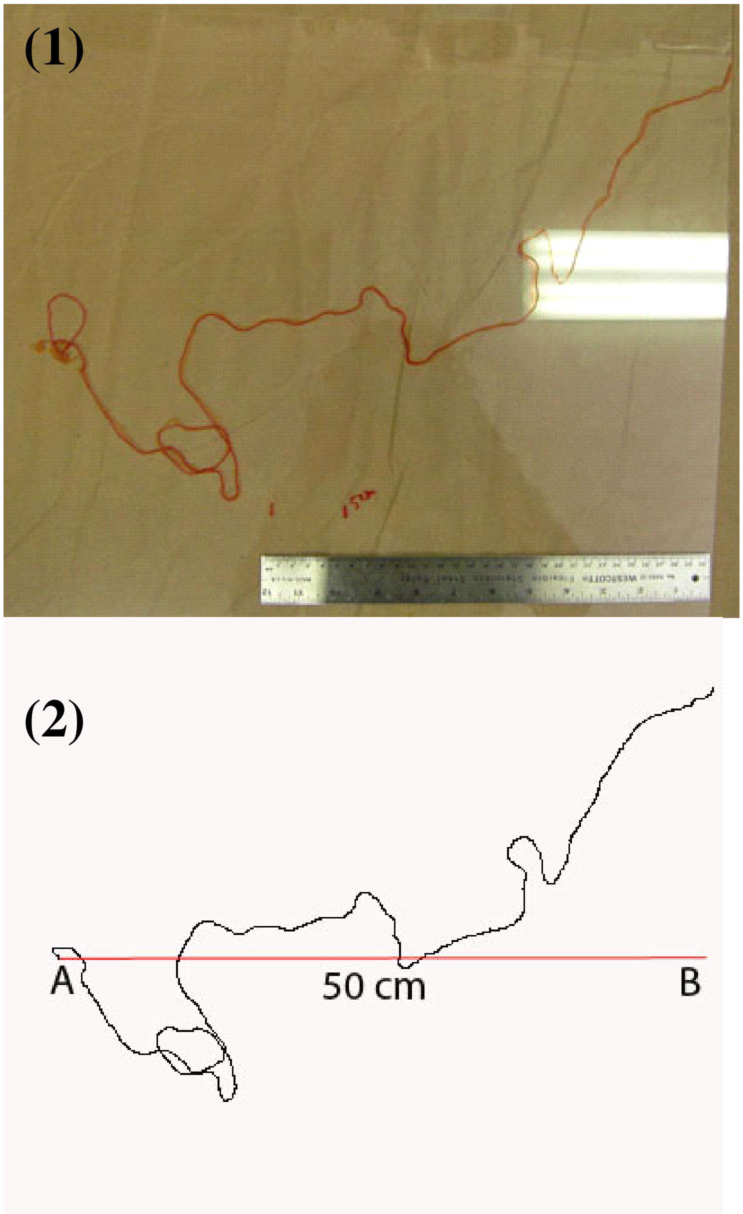

Figure 3-5. (1) Digital photograph taken of a plexiglass sheet showing researcher’s trace of the bed bug movement from 50 cm from the host. (2) Software conversion of bed bug’s movement path from shelter (A), to host (B).

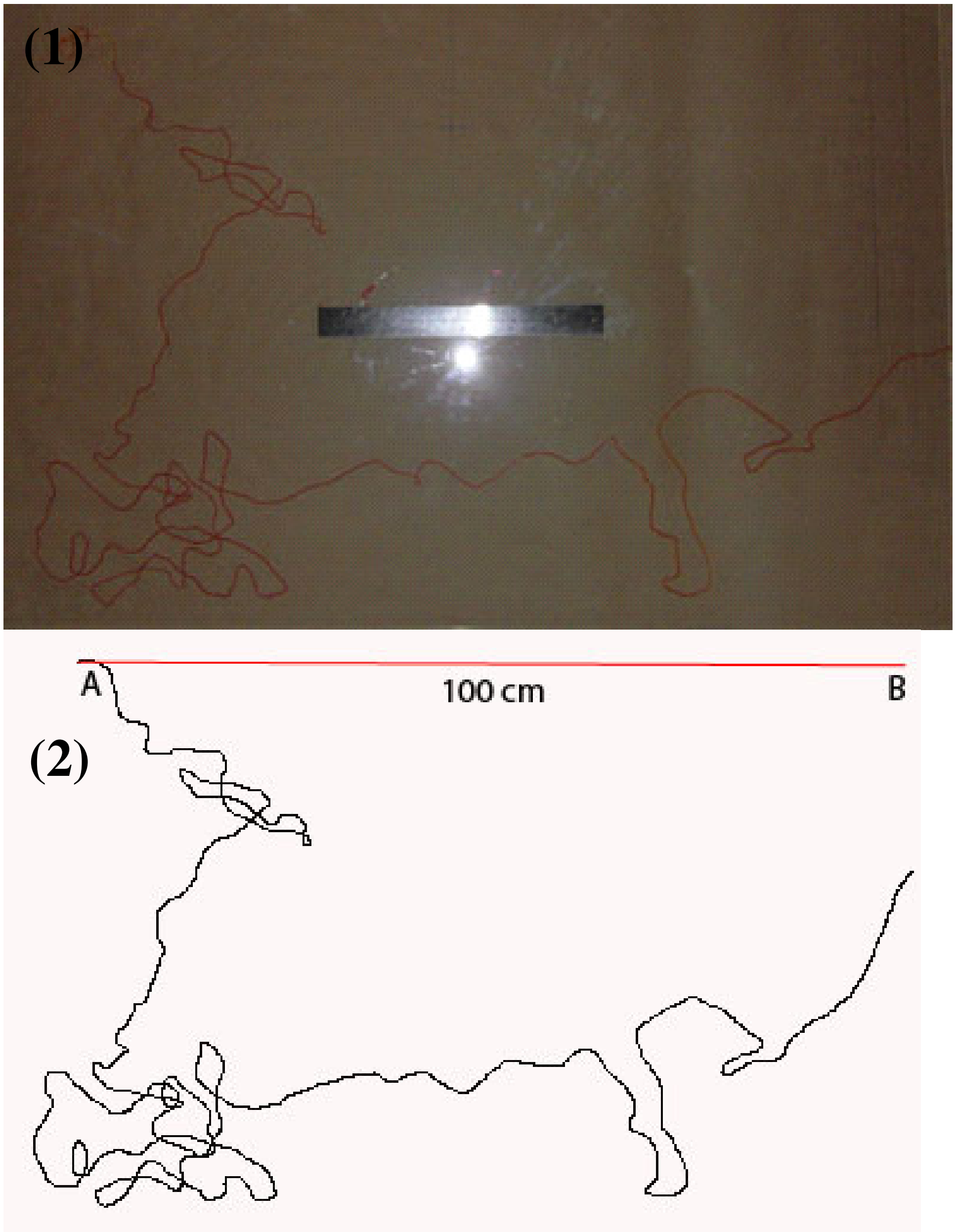

Figure 3-6. (1) Digital photograph taken of a plexiglass sheet showing researcher's trace of the bed bug movement from 100 cm from the host. (2) Software conversion of bed bug's movement path from shelter (A), to host (B).

Chapter 4. An evaluation of the affect of blood feeding on the bed bug's (individual and in groups) response to aggregation cues.

Introduction

The bed bug, *Cimex lectularius* L., was once a public health pest worldwide. It was estimated that in the early 20th century, up to 33% of the homes in London, Britain were infested with bed bugs. In developed nations, bed bug infestations declined through improvements in sanitation and with increased use of residual insecticides such as DDT (Usinger 1966). However, the downward trend in bed bug infestations began to reverse in the 1990s and there have been increased reports of infestations worldwide (Boase 2001).

Traps and monitors have been designed to trap and intercept hungry bed bugs. These traps use host cues such as heat, CO_2, and other chemical odors to attract and capture the hungry bed bugs (Anderson et al. 2009, Wang et al. 2009). Preliminary studies with *Triatoma infestans* Klug have demonstrated how feces and cuticular washing could be applied as lures to traps. In theory, these traps could exploit the *T. infestans* aggregation behavior and capture those individuals that have recently fed and are no longer attracted to host cues (Lorenzo and Lazzari 1996).

The chemical ecology and pheromone communication of bed bugs has been investigated so that these chemicals may be exploited for detection and control of infestations. Many of the chemicals investigated as potential attractants of bed bugs are derived from materials found in the bed bug's aggregations. Bed bugs are attracted to volatiles from the feces of conspecifics (Siljander et al. 2007). They are also known to be attracted to the dichloromethane-soluble elements of fifth instar exuvia (Domingue et al. 2010), and volatiles excreted from metathoratic glands of adult bed bugs (Levinson and Bar Ilan 1971, Siljander et al. 2008). In addition, tactile stimuli from close contact with other bed bugs will also induce a bed bug aggregation behavior.

The feeding status of a blood feeding insect can affect its behavior towards aggregation cues and host sensory cues. Olson (2009) demonstrated that starved bed bugs were not as attracted to feces of conspecifics as those bed bugs that were not starved. On the other hand, Lorenzo (1994) found that the kissing bug, *Triatoma infestans*, did not respond to aggregation cues for 24 hours after feeding. Alternatively, the bed bug will no longer be attracted by host cues for at least five hours after a blood meal (Wintle and Reinhardt 2008).

Although a number of aggregation components have been identified that are attractive to bed bugs, there are still many potential aggregation components to be investigated. In addition, much of the aggregation behavior of the bed bug must be explored before chemical ecology can be applied. The present research examined the aggregation response of an individual bed bug to aggregation materials in dual choice chamber olfactometer. In addition, we also examined if feeding status (fed, unfed) influenced the response of the bed bug to these materials. Finally, the aggregation behavior of a group of recently fed and starved bed bugs towards aggregation cues was examined in arena bioassays.

Materials and Methods

Origin and maintenance of *Cimex lectularius* colony. *Cimex lectularius* were collected from human dwellings in Cincinnati, Ohio. Bed bugs were kept in plastic jars (7 cm x 5 cm) containing a cardboard substrate that provided a surface on which the bed bugs could walk and oviposit. Bed bugs were maintained in an environmental chamber at 23-26^{o}C, 40-70% RH, and a L12:D12 photoperiod. The bed bugs were fed once a week on chicken blood through an artificial membrane system or on human volunteers.

Preparation of Potential Aggregation Attractants (PAA). All PAAs that were tested were placed inside a folded filter paper shelter (2 cm^2; folded in half, Whatman®, Maidstone, England). The PAAs tested were: adult feces, fifth instar exuvia, live adult male bed bugs, and live adult female bed bugs. Bed bug feces were collected by placing four recently fed adult bed bugs (2 males, 2 females) on a filter paper shelter. These bed bugs were left to defecate on their respective shelters for 48 h, after which the bed bugs were removed. To collect exuvia, fifth instar bed bugs were identified under a dissecting microscope and separated into a plastic feeding jar. These bed bugs were fed and allowed to molt. Four fifth instar exuvia were removed and secured to a paper shelter using double sided sticky tape (Scotch®, 3M, St. Paul, MN). To prepare shelters containing a live bed bug as an attractant, one adult bed bug was starved for 7 days and then restrained to a paper shelter using double sided sticky tape. Bed bugs were restrained to filter paper shelters four hours prior to bioassays to ensure the bed bugs were acclimated. All paper shelters containing aggregation attractants were stored in an environmental chamber maintained at 23-26^{o}C, 40-70% RH, and a L12:D12 photoperiod.

Bioassay of individual bed bug. The response of an individual bed bug towards PAAs was evaluated in dual choice chamber olfactometers (Figure 4.1 and 4.2) and square arenas (Figure 4.3). Each olfactometer consisted of two circular glass chambers (each 125 mm x 65 mm) linearly interconnected by plastic tubing (10 cm x 1cm i.d.). PAAs were randomly assigned to one of the two lateral chambers and a filter paper strip (15 cm x 0.5 cm) extended through the plastic tube to facilitate interchamber movement of the bioassayed bed bugs (Figure 4.2). For each experiment one new adult bed bug was released in the connecting tube 30 minutes into scotophase. A bed bug was classified as a responder when it was found 16 hours later in the one of the glass chambers or resting on the filter paper shelter containing the PAA. After each replicate, olfactometers were washed thoroughly with hot water and detergent (Sparkleen™ 1, Fisher Scientific Co., Pittsburgh, PA) and allowed to air dry.

Bioassay of bed bug groups. To test the response of a group of bed bugs to PAAs, bed bugs were placed in square arenas (Figure 4.3). The square arenas were 24 cm^2 and the floor was covered with brown kraft paper (40#, Westrick Paper Company, Jacksonville, FL). The walls of the arena were 2 cm tall and were coated with Fluon® (AGC Chemicals Americas, Inc., Exton, PA) and a lid with three large ventilation holes was placed over the arena to prevent the bed bugs from escaping. A filter paper shelter containing a PAA was placed inside the arena, 8 cm from the arena's center. In the center of the arena was placed 10 bed bugs of the same sex. The bed bugs could choose to aggregate together in the shelter or anywhere else in the arena. After each replication, the kraft paper covering the floor of the arena was replaced.

To compare the effectiveness of test stimuli, both olfactometer and arena experiments were often run in parallel to minimize the effect of any confounding variables, such as barometric pressure or humidity (Figure 4.3). All experiments were conducted at 22-26°C, 25-40% RH, under a L12:D12 photoperiod.

Statistical analysis. The mean number of bed bugs responding to PAAs in the two-chambered olfactometer and arena experiments were analyzed using the χ^2 goodness-of-fit test ($\alpha = 0.05$) (Zar 2009). The mean percentage of bed bugs that aggregated in the test shelter versus another location in the arena was analyzed with the Wilcoxon paired-sample test ($\alpha = 0.05$) (Zar 2009).

Results

Response of individual bed bugs to PAAs. Regardless of the sex or feeding status of the bed bug, individuals were found in the chamber that contained feces more often than the control chamber (Figure 4.4). Similarly, individuals were found more often in the chamber that contained exuvia than in the control chamber, regardless of the sex or feeding status of the bed bug (Figure 4.5).

Figure 4.6 summarizes an individual bed bugs response towards chambers containing a single male bed bug as a PAA. Males that were starved for seven days were found more often in a chamber containing a single male bed bug ($\chi^2 = 4$; df = 1; $P = 0.0455$). Alternatively, females that were starved for seven days were found more often in the control chamber ($\chi^2 = 4$; df = 1; $P = 0.0455$). Recently fed male and female bed bugs were found just as often in the test chamber as in the control chamber.

Males that had been starved for seven days and recently fed males were found more often in a chamber containing a single female bed bug as a PAA than in a control chamber ($\chi^2 = 7$; df = 1; $P = 0.0093$ and $\chi^2 = 4$; df = 1; $P = 0.0455$; respectively) (Figure 4.7). Alternatively, female bed bugs starved for seven days were found more often in the control chamber ($\chi^2 = 4$; df = 1; $P = 0.0455$). Recently fed female bed bugs were found in the control chamber just as often as the test chamber.

Bed bug group-response to aggregation cues. Groups of bed bugs, regardless of sex and feeding status, were found more often aggregated in shelters containing feces as a PAA (Figure 4.8). Similarly, groups of bed bugs, regardless of sex and feeding status, were found more often in shelters containing exuvia as a PAA (Figure 4.9).

Figure 4.10 summarizes the aggregation response of a group of bed bugs towards a shelter containing a single male bed bug as the PAA. A group of bed bugs that were starved for seven days, regardless of sex, were found more often aggregated in the arena than in the shelter containing the male bed bug as the PAA. In addition, recently fed males and females were also found aggregated in the arena more often than in the shelter containing the male bed bug as the PAA.

Both males and females, starved for 7 days, were found aggregated in the arena more often than in the shelter containing a female bed bug as the PAA (Figure 4.11). In addition recently fed males and females were found aggregated in the arena more often than in the shelter containing the female bed bug as the PAA.

Discussion

In both individual and group bioassays, the feces of conspecifics were attractive to all bed bugs regardless of feeding status. Feces had been previously reported as an attractant for unfed bed bugs (Siljander et al. 2007, Olson et al. 2009). Bed bugs are attracted to feces because large accumulations of feces are indicative of low levels of disturbance and offer protection from predators (Lorenzo and Lazzari 1996). In addition, preference for shelters containing feces would also bring the bed bugs to places where conspecifics and mates were available. The blood feeding Hemipteran, *T. infestans*, will seek out crevices and other protected areas during the day. *T. infestans* will use feces as a chemical mark at the entrance of a refuge, helping other *T. infestans* to find the refuge and other conspecifics (Lorenzo and Lazzari 1996).

The bed bugs exuvia was also observed to be attractive to both male and female bed bugs regardless of feeding status. Interestingly, Domingue (2010) determined that only male bed bugs were attracted to extracted dichloromethane soluble elements of fifth instar exuvia. The attractiveness of fifth instar exuvia could be attributed to three physiological factors: (1) pheromone residues from scent glands located on the dorsal region of fifth instars that are shed with the exoskeleton, (2) traces of feces located in the hind gut cuticle after molting, and/or (3) tactile stimuli caused by the physical presence of the exuvia. Figueira (1994) also reported that *T. infestans* was attracted to hexane-soluble elements of fifth instar *T. infestans* exuvia. In addition, Figueira (2009) found in preliminary studies, traps containing cuticular extracts enhanced aggregation response and suggested the potential utility of these extracts for insect detection. Similar to the response of bed bugs to feces, large numbers of exuvia could attract bed bugs to areas where conspecifics are available. An accumulation of many exuvia could also indicate an area of protection and minimal disturbance.

In the individual bioassays, both starved and fed male bed bugs exhibited preference for chambers containing a female bed bug. The fed male bed bugs attraction towards a female bed bug was not surprising because Stutt (2001) found that the majority of the copulation bouts of male bed bugs occurred within 36 hours of a blood meal. Recently fed males would be attracted to the chambers containing a female because of an increased drive to mate. Our observations of male bed bug behavior support an earlier study by Siljander (2008) which reported that male bed bugs were attracted to "airborne aggregation pheromones" collected from the headspace of a jar containing male and female bed bugs.

In individual bioassays, both recently fed and starved females were not significantly attracted to chambers containing a male or a female bed bug as an attractant. These observations appear to conflict with the results of a study conducted by Siljander (2008), where females were attracted to volatiles released by conspecifics. However our experimental design was different from the one used by Siljander. It could be argued that the females may not have been attracted because of alarm pheromones released by the restrained bed bug within the shelter. The almost even distribution of recently fed females in both chambers suggests that they were neither attracted nor repulsed by the confined conspecific. Additionally, there was evidence that the recently fed males were attracted to chambers containing bed bugs restrained in a similar fashion. It appears that the attractive qualities of a single live bed bug are produced by both male and female bed bugs; however female bed bugs are unresponsive towards it.

In all group bioassays, fed and unfed bed bugs (male and female) did not aggregate in the shelter containing a single conspecific. In fact, there was a tendency to avoid the shelter containing the restrained bed bug based on the statistical analysis. While a superficial examination of the data might suggest that the bed bugs were detecting an alarm pheromone, the individual assay results revealed that individual bed bugs were attracted to a confined conspecific. Instead, bed bugs in the group bioassays are choosing to aggregate with each other and may not be contacting the shelter before coming to rest in some other location within the arena.

The group bioassays demonstrated how both fed and unfed bed bugs, within an arena, can use feces or exuvia to locate and aggregate with conspecifics. *T. infestans* have been observed to defecate around the access of harborages and other conspecifics of *T. infestans* are able to locate the refuge because they are attracted to the feces (Lorenzo and Lazzari 1996). We suggest, that similar to *T. infestans*, that bed bugs use feces as a chemical mark that helps other bed bugs to locate a refuge.

Our data indicate that bed bugs that are starved for seven days and bed bugs that have recently fed are attracted to feces and exuvia. However, starved and recently fed males were found significantly more often in a chamber containing a female bed bug than in control chambers. The arena bioassays demonstrated how a simple filter paper shelter containing feces or exuvia was attractive to a group of bed bugs. Future focus should be placed on chemically characterizing feces and exuvia for their potential use for population sampling and trapping of starved and recently fed bed bugs in human dwellings.

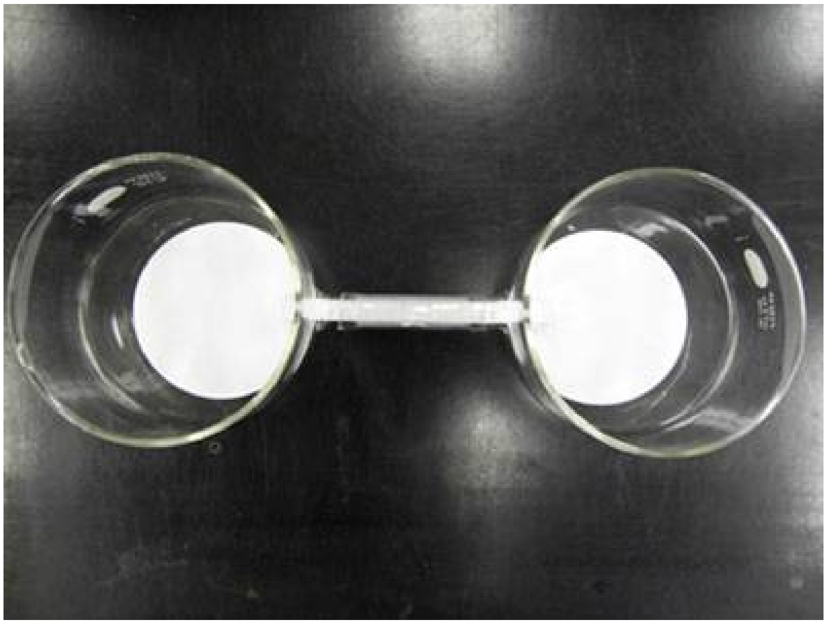

Figure 4-1. Dual choice chamber olfactometer used to determine an individual bed bug's response to a potential aggregation attractant.

Figure 4-2. Tubing used to connect chambers of the olfactometer. Filter paper was placed inside the tube to facilitate interchamber movement of bed bugs.

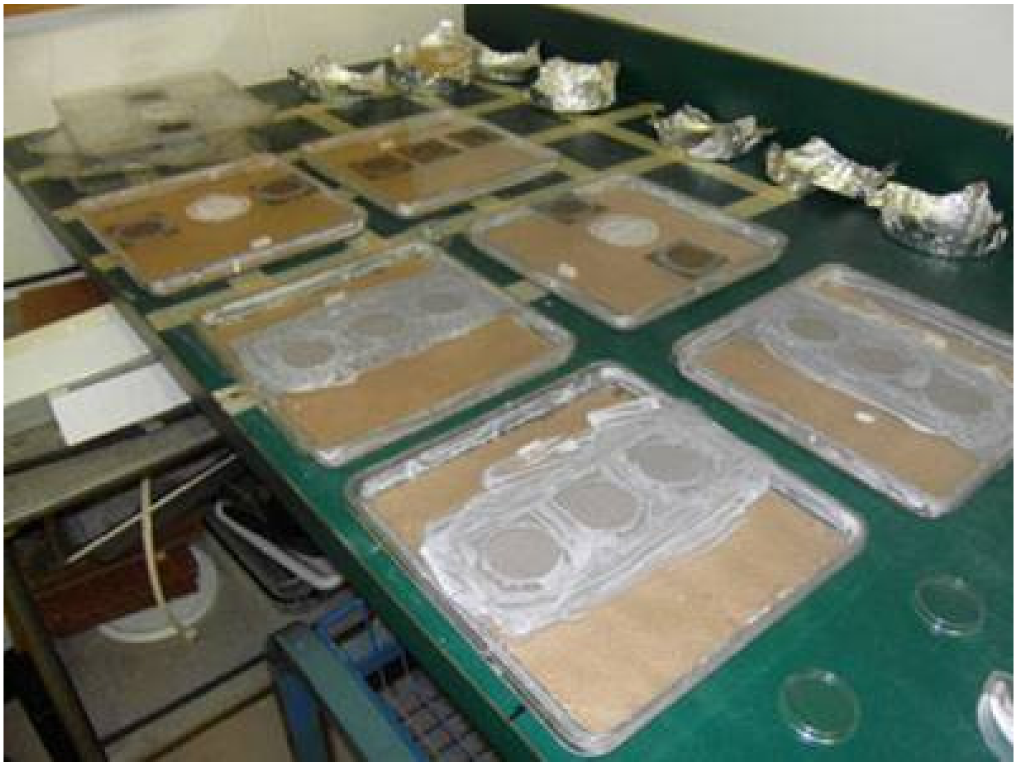

Figure 4-3. Square arena used to determine the response of a group of 10 bed bugs to a potential aggregation material. Arena experiments were often run on the same night to minimize the effect of any confounding factors, such as barometric pressure or humidity.

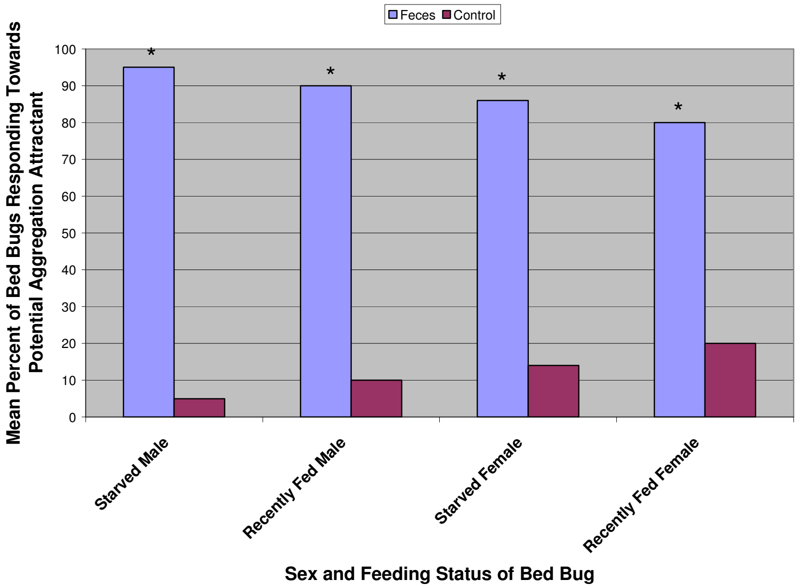

Figure 4-4. Mean percent of starved (7 days) or recently fed bed bugs (male or female) in chambers with or without feces as a potential aggregation attractant. An asterisk indicates a significant response to the treatment; χ^2 test, α = 0.05. n=20.

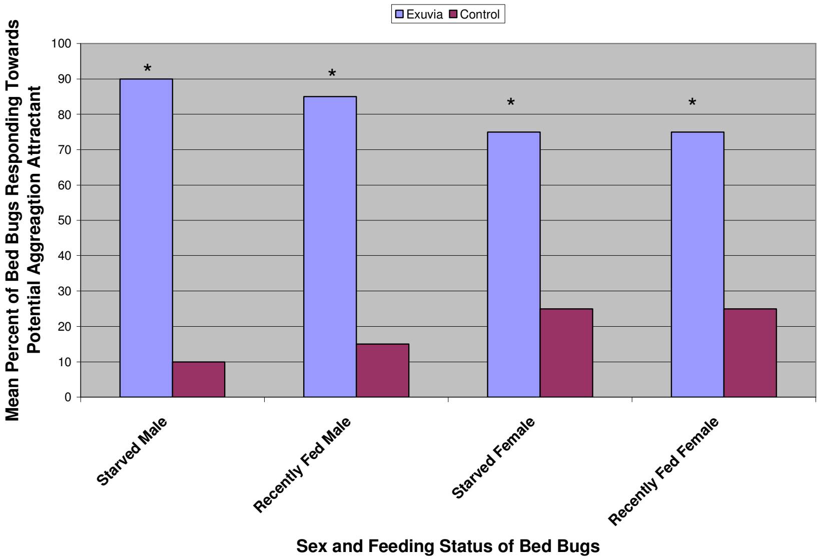

Figure 4-5. Mean percent of starved (7 days) or recently fed bed bugs (male or female) in chambers with or without exuvia as a potential aggregation attractant. An asterisk indicates a significant response to the treatment; χ^2 test, α = 0.05. n=20.

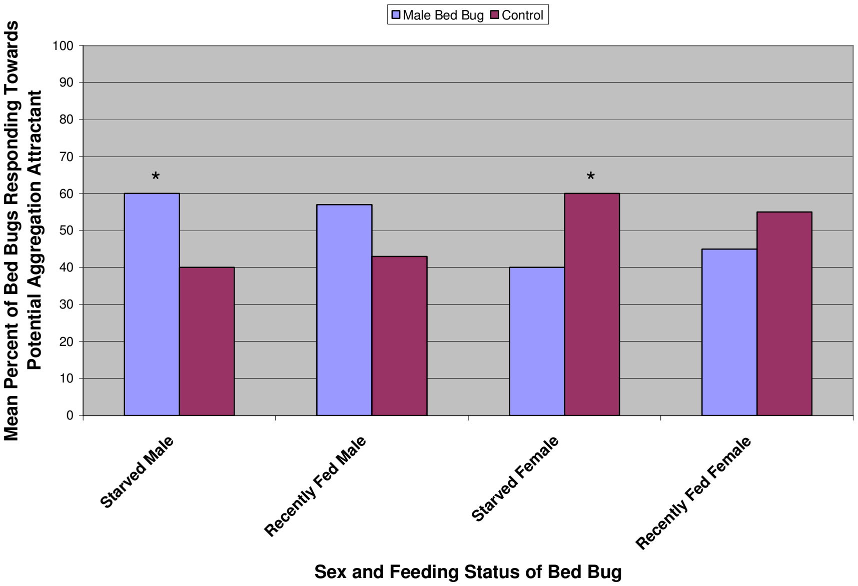

Figure 4-6. Mean percent of starved (7 days) or recently fed bed bugs (male or female) in chambers with or without a single male bed bug as a potential aggregation attractant. An asterisk indicates a significant response to the treatment; χ^2 test, $\alpha = 0.05$. n=20.

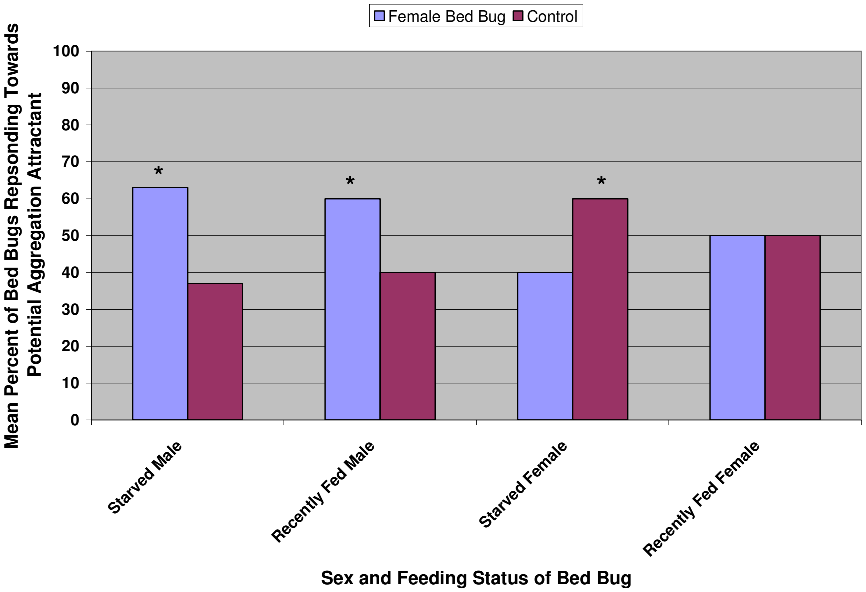

Figure 4-7. Mean percent of starved (7 days) or recently fed bed bugs (male or female) in chambers with or without a single female bed bug as a potential aggregation attractant. An asterisk indicates a significant response to the treatment; χ^2 test, $\alpha = 0.05$. n=20.

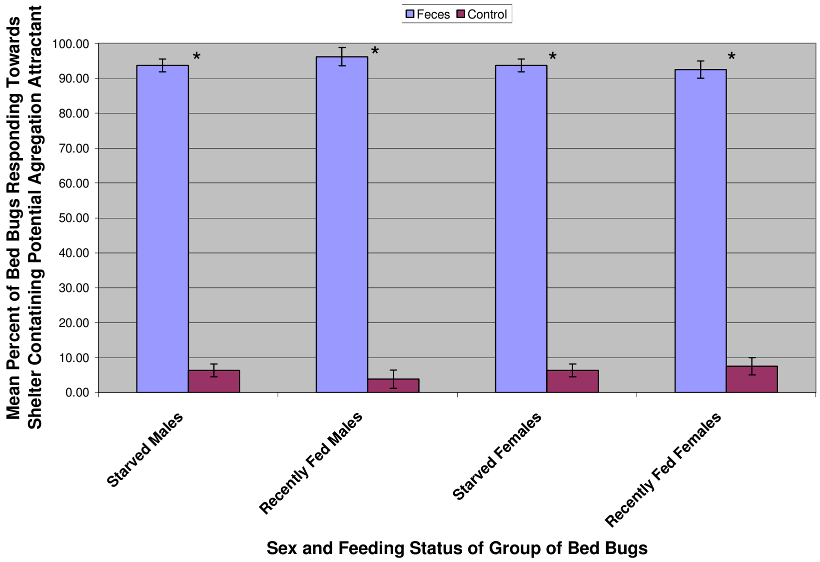

Figure 4-8. Mean (±SE) percent of 10 starved (7 days) or recently fed bed bugs (male or female) aggregated outside or within a paper shelter containing feces as a potential aggregation attractant. An asterisk indicates a significant response to the treatment; Wilcoxon paired-sample test; α = 0.05, n=8.

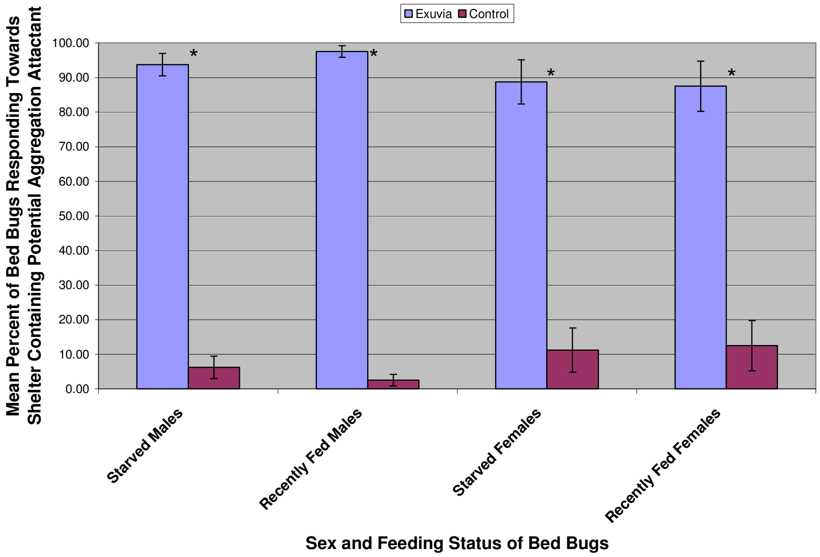

Figure 4-9. Mean (±SE) percent of 10 starved (7 days) or recently fed bed bugs (male or female) aggregated outside or within a paper shelter containing exuvia as a potential aggregation attractant. An asterisk indicates a significant response to the treatment; Wilcoxon paired-sample test; α = 0.05, n=8.

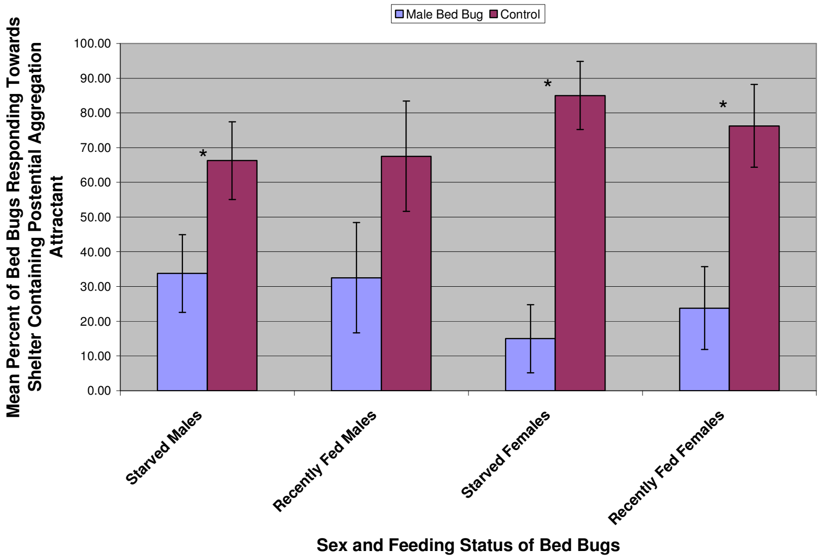

Figure 4-10. Mean (±SE) percent of 10 starved (7 days) or recently fed bed bugs (male or female) aggregated outside or within a paper shelter containing a single male bed bug as a potential aggregation attractant. An asterisk indicates a significant response to the treatment; Wilcoxon paired-sample test; α = 0.05, n=8.

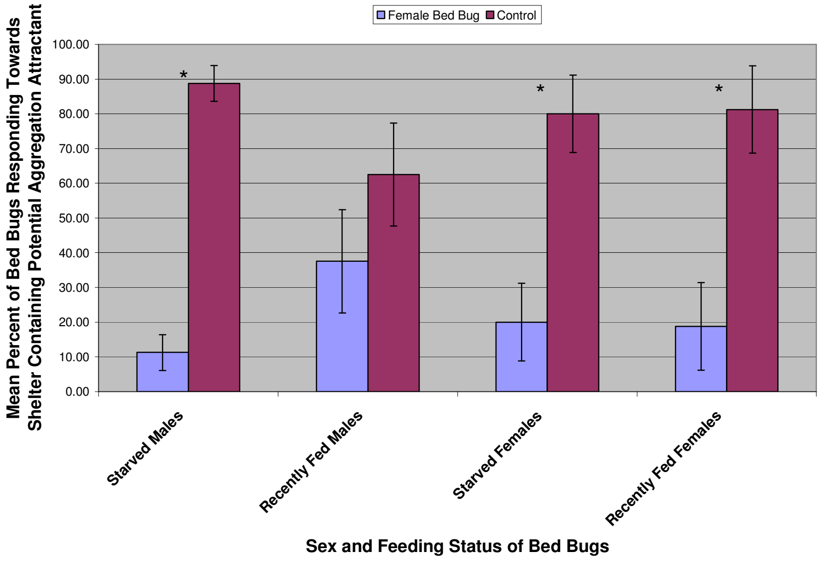

Figure 4-11. Mean (±SE) percent of 10 starved (7 days) or recently fed bed bugs (male or female) aggregated outside or within a paper shelter containing a single female bed bug as a potential aggregation attractant. An asterisk indicates a significant response to the treatment; Wilcoxon paired-sample test; α = 0.05, n=8.

Chapter 5. Host searching and aggregation activity of recently fed and unfed bed bugs (*Cimex lectularius* L.)

Introduction

The bed bug, *Cimex lectularius*, is a member of the family Cimicidae. This family is composed entirely of blood feeding ectoparasites that feed on hosts that include bats, birds, and humans (Ryckman et al. 1981). The bed bug feeds primarily on humans and requires several blood meals in order to reach adulthood. As an adult, the bed bug requires additional blood meals in order to reproduce (Johnson 1941).

The bed bug exhibits a nocturnal feeding behavior (Mellanby 1939). Periods of peak bed bug activity occur between the hours of 3:00 am and 6:00 am. During the daylight hours bed bugs remain hidden together in cracks and crevices. These aggregations of bed bugs are located in areas of low disturbance (Usinger 1966). Aggregating in groups with conspecifics presents advantages for the bed bug which include the decreased chance of desiccation and the increased chance of encountering a mate (Benoit et al. 2007, Siljander et al. 2008).

The feeding status of a blood feeding insect will affect its circadian rhythm. Blood-feeding Hemipterans in the taxa Triatominae will synchronize several activities, such as searching for a host, with environmental light-dark cycles. Using actographs, machines that measure activity, the daily rhythm of a confined *Triatoma infestans* Klug in the absence of a host has been described as having two peaks of activity (bimodal activity), one at dusk and one at dawn (Lazzari 1992). However when a regular host is present, *T. infestans* exhibits unimodal activity, leaving its shelter the first hour of scotophase and returning prior to photophase (Lorenzo and Lazzari 1998). In addition, prolonged starvation will cause increased searching activity in *T. infestans*. The feeding status of a bed bug has been shown to affect its attraction towards aggregation cues. Olson (2009) reported that the bed bug's attraction towards the feces of conspecifics diminished with time after feeding. In addition, the feeding status of a bed bug can affect its attraction towards a host. For example, bed bugs that have recently fed are repulsed by the same sensory cues that attracted them to the vicinity of the host (Usinger 1966).

Understanding how a bloodmeal affects the bed bug's activity and aggregation behavior within a 24 hr period could aid in monitoring strategies for bed bug infestations. Bed bugs that have recently fed will not be attracted towards host cues. Alternatively, starved bed bugs may be attracted towards host-sensory cues but only during certain periods during a 24hr period. Although a great amount of recent work has focused on the hungry bed bugs attraction towards a host, little research has focused on the movement and aggregation behavior of recently fed bed bugs. However, because bed bugs feed every 5-7 days, the majority of the population on any given day is not feeding (provided a host is present regularly). Therefore, very few bed bugs relative to the population will be attracted to traps or other host sensory cues.

The purpose of this study was to investigate the aggregation behavior of bed bugs with regard to an established aggregation location. The following experiments were intended to determine the circadian activity patterns of two bed bug groups: those that successfully fed on a host and those that did not. Circadian activity between single sex groups of males and virgin females were also determined.

Material and Methods

Origin and maintenance of *Cimex lectularius* colony. Bed bugs (*Cimex lectularius* L.) were collected in 2008 from a hotel in downtown Cincinnati, Ohio and reared at the Dodson Urban Pest Management Laboratory in Blacksburg, Virginia. These bed bugs were maintained in an environmental chamber at 23-26^{o}C, 40-70% r.h. and 12L/12D photoperiod. The bed bugs were kept in plastic rearing jars containing folded cardboard paper. The cardboard paper was added to the rearing jars to provide the bed bugs with a surface on which to oviposit and walk. The tops of the plastic rearing jars were covered with a nylon mesh. The nylon mesh allowed for the bed bugs to be fed without removing them from the jars. Bed bugs were fed once a week on chicken blood treated with sodium citrate. The chicken blood was heated to a temperature of 36-38^{o}C using a hot water bath and water circulation system.

Preparation of bed bugs. Each replication consisted of ten bed bugs of the same sex; however, both male and virgin female bed bug aggregation behavior was observed and quantified. Virgin female bed bugs were obtained by separating fifth instar bed bugs individually into vials. Each vial containing a fifth instar bed bug was fed and the females were collected as soon as they molted from the fifth instar. In each experiment, ten bed bugs of the same sex were fed and placed on a 2 cm^2 shelter made of filter paper folded once (Whatman® #1, Maidstone, England). These bed bugs were then left on their respective shelter to starve for 14-21 days. After the bed bugs had been starved, the bed bugs and their respective shelter were placed inside a test arena approximately 3-4 cm from the arena center.

Bioassay arena. The arena used to observe the aggregation behavior of bed bugs consisted of a plastic dish (24 cm^2, 2 cm walls, Nunc^{tm}, Rochester, NY) with its bottom covered with brown kraft paper (40#, Westrick Paper Company, Jacksonville, FL). The sides of the arena were coated with fluon (AGC Chemicals Americas, Inc., Exton, PA) to prevent bed bugs from escaping. An artificial feeder containing heated chicken blood was then placed directly over the arena. The bed bugs would leave their respective shelter when the heat from the chicken blood in the artificial feeder was detected.

Induction of aggregation behavior. The aggregation behavior of both fed and unfed bed bugs was observed. One group of bed bugs was provided with a filter paper tab hanging from the bottom of the artificial feeder that provided the bed bugs access to climb up and obtain a blood meal (Figure 5.1A). These bed bugs were given 30 minutes to feed to repletion and drop off of the feeder. The second group of bed bugs was not provided with a paper tab suspended from the artificial feeder (Figure 5.1B). Therefore, these bed bugs could forage, but could not obtain a blood meal. After 30 minutes, the artificial feeder was removed and a digital video camera (Sony DCR-TRV250 Digital Video Recorder, Sony®) was placed over the arena. The bed bugs behavior was captured from 9:00 pm to 11:00 am. Environmental conditions within the room were maintained at 26-27^{o}C, 20-40% r.h., and a 12L:12D photoperiod.

Statistical analysis. The aggregation behavior of fed and unfed bed bugs was analyzed using an Analysis of Variance (ANOVA). In addition, all variables were compared using Tukey-Kramer HSD to determine if the means were significantly different ($\alpha = 0.05$). Values used to quantify aggregation behavior were from 9:30 pm - 6:00 am. Values two hours prior to photophase were not included in statistical analysis.

These values were not used because both unfed and fed bed bugs may be influenced by an innate circadian rhythm. All statistical procedures were calculated with the software package JMP®8 (JMP8 2008).

Results

After the artificial feeder was detected, bed bugs would leave the shelter to forage. The majority of male bed bugs (≈ 70%) that fed were observed to return immediately to a shelter (Figure 2). Similar to male bed bugs, the majority of virgin female bed bugs (≈75%) that fed also returned immediately to a shelter to aggregate (Figure 3). However, male bed bugs (≈ 25%) that did not feed continued to forage for 7-8 hours throughout the night (Figure 5.2). Virgin female bed bugs that did not feed exhibited similar behavior as unfed male bed bugs and continued to forage for 6-8 hours throughout the night (Figure 5.3). However, at approximately 4:00-6:00 am, the remaining fed and unfed bed bugs of both sexes began to migrate towards a shelter to aggregate such that 70-95% of the bed bugs had aggregated before the onset of photophase (Figure 5.2 and 5.3).

Comparison of male and female bed bug aggregation behavior. There was no significant interaction between sex and feeding status of the bed bug (d.f. = 3, P= 0.6344. The majority of male and virgin female bed bugs (66.3% and 71.1%; respectively) returned to a shelter after a blood meal. In addition, the mean percent of unfed males and virgin female bed bugs aggregating between the hours of 9:30 pm and 6:00 am was found not to be statistically significant.

Male bed bug aggregation behavior. The aggregation behavior of male bed bugs is summarized in Table 5.1. A significantly larger percentage of fed male bed bugs were observed aggregated compared to the proportion of unfed male bed bugs observed aggregated. The mean percentage of fed male bed bugs that were observed aggregated within the shelter was 66.3 ± 1.8%. The mean percentage of unfed male bed bugs that were observed aggregated within a shelter was 16.9 ± 1.8%.

Virgin female bed bug aggregation behavior. The aggregation behavior of female bed bugs is summarized in Table 5.1. Similarly to male bed bug aggregation behavior, a significantly larger percentage of fed virgin female bed bugs were observed aggregated compared to the percentage of unfed virgin female bed bugs observed aggregated. The mean percentage of recently fed virgin female bed bugs that were observed aggregated within the shelter after a blood meal was 71.7 ± 1.8%. The mean percentage of hungry virgin female bed bugs that were observed aggregated within the shelter was 20.6 ± 1.8%.

Discussion

These experiments demonstrated that most foraging bed bugs leave their respective shelter, locate a host, feed, and return back to a shelter in a relatively short period of time (≈ 70% in 30 minutes). However, bed bugs that leave the shelter and do not obtain a blood meal may continue to search for a host for many hours (≈ 70% were not aggregated and were searching between 11:30 pm and 4:00 am) before they return to a shelter.

Fed bed bugs immediately returned (within 30 minutes) to a shelter to aggregate with conspecifics. The fed bed bugs' aggregation behavior can be explained by a number of factors: 1) the bed bug is fed and is no longer stimulated to search and feed on a host but stimulated to escape and harborage (Wintle and Reinhardt 2008), 2) the bed bug seeks out a harborage to aggregate with conspecifics and reduce desiccation (Benoit et al. 2007), 3) bed bugs return to the shelter in order to locate a mate.

Bed bugs that were unable to obtain a blood male did not immediately aggregate but continued to move and search throughout the arena for a host. Lehane (2005) explained host location in three steps: appetitive searching that is driven by hunger and often occurs at specific times; activation; where oriented behavior is exhibited; and attraction, where a host stimuli brings the insect into contact with the host. The first step, appetitive searching, could explain why the hungry bed bugs continued searching throughout the night. The initial presence of a stimuli triggered searching behavior. This searching behavior continued throughout the night until approximately 5:30 am. After 5:30 am the bed bugs began to move towards the shelter and aggregate with conspecifics.

The foraging and searching activity we observed of unfed bed bugs is similar to observations made by Mellanby (1939), who observed that peak bed bug foraging activity occurred between the hours of 3:00 am and 5:30 am. However, our results indicated that unfed bed bugs were active over a longer period of time with peak foraging activity occurring between 11:30 pm and 6:00 am. Our results could differ because Mellanby (1939) observed a population of bed bugs in the field while our colony had been acclimated to conditions within an environmental chamber for 2 years. In addition, the feeding status of the bed bugs observed in the Mellanby (1939) study was not specified and most likely unknown.

Starved bed bugs exhibited more activity than bed bugs that had recently fed. How (2010) reported similar results when observing the tropical bed bug, *Cimex hemipterus* Fabricius. The hungry tropical bed bug of all life stages exhibited more searching movement and activity than tropical bed bugs that were recently fed with the exception of recently fed female tropical bed bugs. While the movement activity of tropical bed bugs decreased after a blood meal, the movement activity of fed female tropical bed bugs did not. However, the mating status of the recently fed female *C. hemipterus* was not specified in the How (2010) report. It has been documented that mating status of the female bed bug, *C. lectularius*, affects the female movement activity (Siljander et al. 2007, Siljander et al. 2008, Pfiester et al. 2009). Mated females are observed to move away from aggregations containing a high number of male bed bugs. It has also been suggested that mated females initiate the spread of bed bug infestations through dispersive behavior (Pfiester et al. 2009). All female bed bugs used in our experiments were virgin females and had not mated, which could explain why female insects used in our study differed from the observations of How (2010).

A circadian rhythm appears to strongly influence the behavior of both fed and unfed bed bugs. The unfed bed bugs continued to search for a host for 7-8 hours throughout the night. However, both unfed male and virgin female bed bugs were observed to move into the shelter approximately 2 hours prior to photophase at 8:00 am. The bed bugs favored areas of low light and the remaining bed bugs may have returned to the shelter because of its protection from direct light (Aboul-Nasr and Erakey 1968).

The movement activity exhibited by unfed bed bugs was similar to the movement activity of *T. infestans*. For example, Lazzari (1992) observed that *T. infestans* would begin to forage for a host 2 hours into scotophase and after unsuccessfully locating a host would seek refuge 1 hour prior to photophase, when *T. infestans* was reared under a 12L:12D photoperiod. However, the fed bed bugs' movement into shelters containing conspecifics is not similar to those movements of recently fed *T. infestans*. Although, *T. infestans* will return to an aggregation of conspecifics after a blood meal, it will not do so until at least 8 hours after the blood meal. Not only does *T. infestans* move very poorly when ingested with blood and will remain motionless, but it must also eliminate excess fluid. After the blood meal, *T. infestans* eliminates a great amount of urine (40-50% of ingested fluid) within 5 hours before returning to an aggregation (Wigglesworth 1931). Recently fed bed bugs were very mobile and would return to a shelter within 30 minutes where they would then remain motionless. However, bed bugs were observed defecating on and around the shelter after a blood meal.

Our results add new data to the knowledge of the activity patterns of bed bugs and uphold previous observations made of bed bug behavior. For example, after a bed bug has fed it is no longer stimulated to search for a host but immediately returns to an aggregation. The activity patterns of unfed bed bugs in our arena are similar to observations of field populations made by Mellanby (1939). Control of bed bugs might be improved if available techniques could be adapted to exploit these periods of maximum activity.

Table 5-1. Comparison of the average number of a group of male or virgin female bed bugs aggregated within an established shelter located within the bioassay arena.

Sex	Condition after introduction of artificial host	Mean percent of bed bugs aggregated (±SE)
Male	Fed	66.3 ± 1.8 A
Male	Unfed	16.9 ± 1.8 B
Female	Fed	71.7 ± 1.8 A
Female	Unfed	20.6 ± 1.8 B
ANOVA *F* value		275.9

Data are the mean (± SE) percent of male or female bed bugs aggregated within an established shelter every 30 minutes from 9:30 pm – 6:00 am, n=5. Fed and unfed conditions of both sexes were compared. ANOVA was used to indicate statistical significance, d.f. = 3 and $\alpha = 0.05$. Data followed by the same letter are not different at $\alpha = 0.05$ by using Tukey HSD tests.

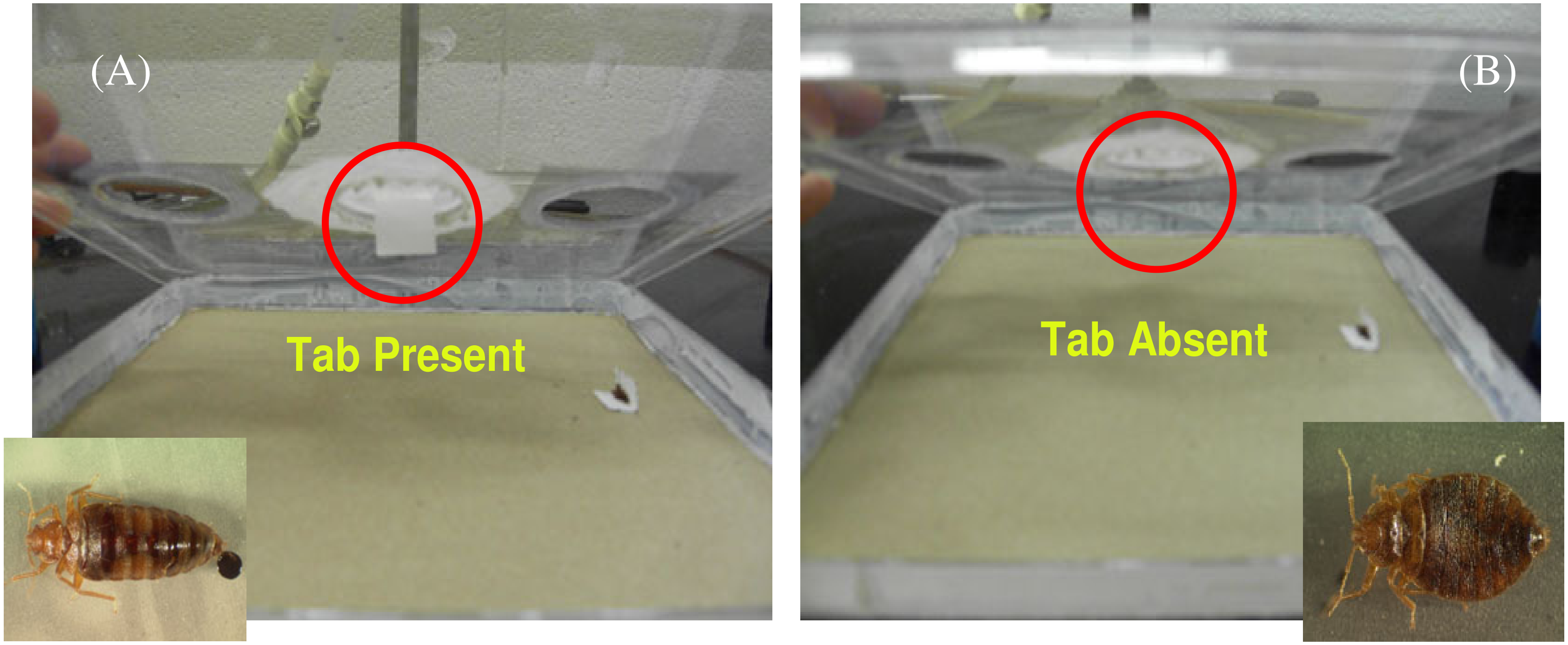

Figure 5-1. Foraging arenas to assess bed bug movement activity. (A) Arena where bed bugs in a paper shelter are able to climb up a paper tab attached to the lid of an arena to reach an artificial feeder. (B) An arena where paper tab is absent so bed bugs in the shelter can detect the feeder but cannot reach it to feed.

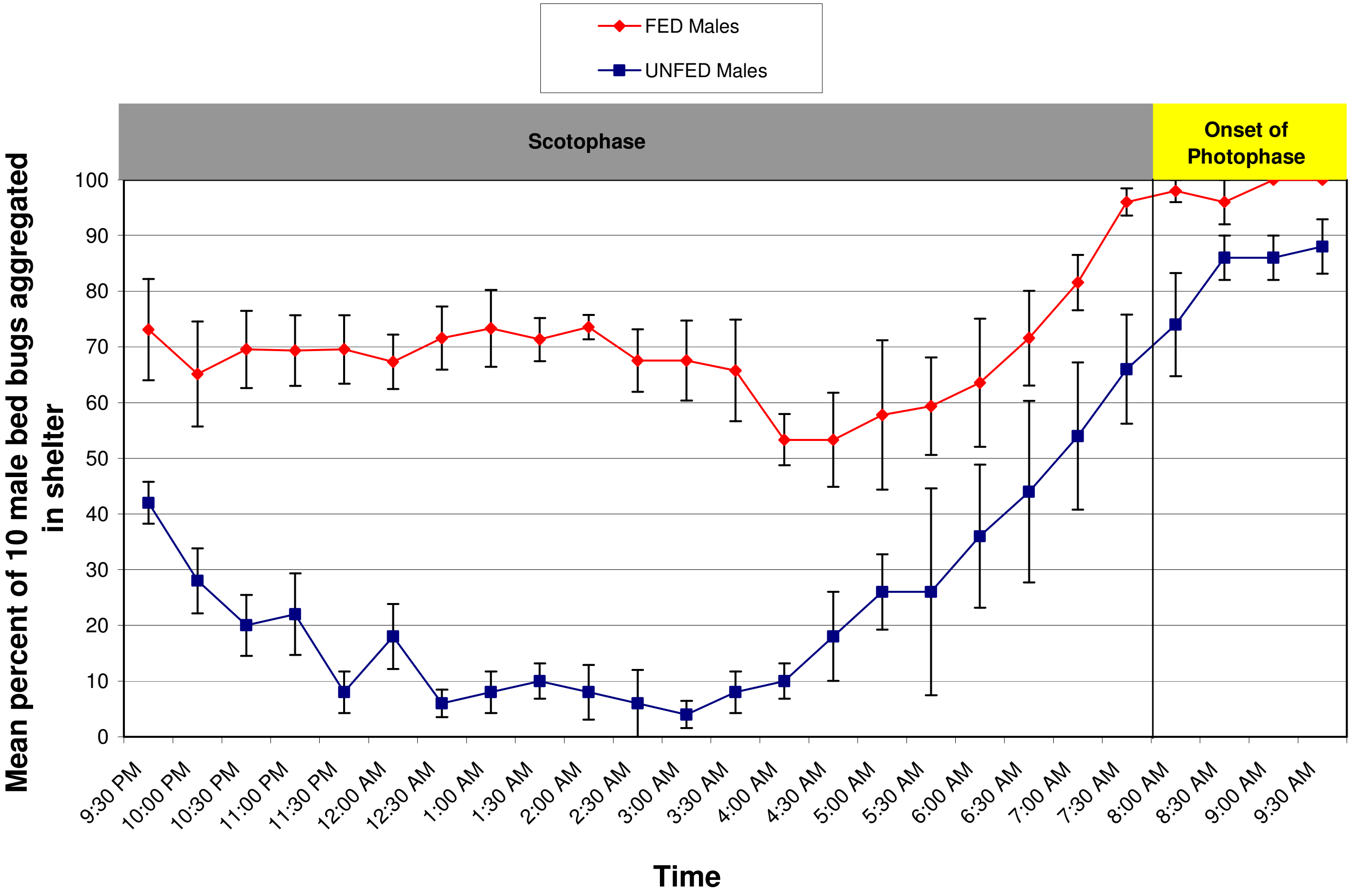

Figure 5-2. Mean percent (±SE) of 10 male bed bugs (fed, unfed) aggregated in harborages after being exposed to an artificial feeder for 30 minutes. n=5 for each treatment group replicate.

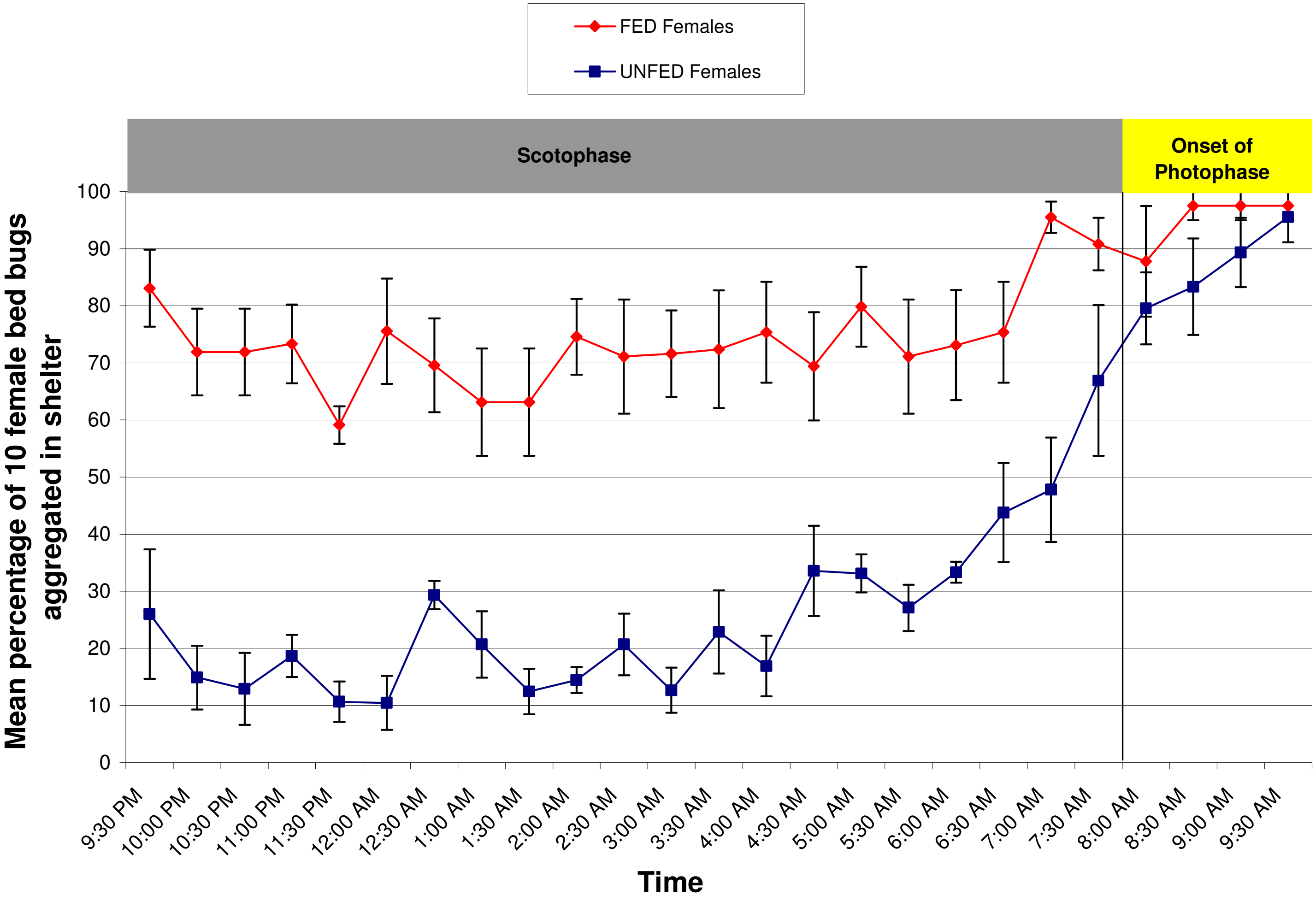

Figure 5-3. Mean percent (±SE) of 10 virgin female bed bugs (fed, unfed) aggregated in harborages after being exposed to an artificial feeder for 30 minutes. n=5 for each treatment.

Chapter 6. Conclusions

The common bed bug is a nocturnal blood feeding parasite of humans, birds, and bats. The bed bug's cohabitation with humans over the last 35,000 years, has led to their world-wide distribution. In the 1900's bed bugs were common household pests in the United States. However, bed bugs were all but eradicated in the US during the 1940 - 50s due to the liberal use of insecticides such as DDT and malathion. Since the 1990s, bed bug infestations have been on the increase and as of 2010, bed bug infestations have been reported in all fifty states.

The primary reason that bed bugs are so undesirable is because of their blood feeding behavior that results in the victim often being covered in itching bites for several weeks. Bed bugs are difficult to control because of their cryptic nocturnal behavior, and their ability to hide in cracks and crevices where they are difficult to locate and treat with insecticides. Little is known about how bed bugs hiding in cracks and crevices are able to locate a host, or what they do after they have taken a blood meal. Several recent studies have focused on bed bug host location and aggregation behavior. However, these studies have been primarily conducted with starved bed bugs that are actively seeking a host. What has been over-looked in many of these studies is the fact that most individuals in a given field population have fed at some point within the last seven days or are in a digestive state. Very little is known about the nocturnal activities of recently fed bed bugs or how these individuals respond to host or aggregation cues.

This study attempted to evaluate the bed bug's response to host and aggregation cues. Both fed and unfed bed bugs were evaluated to determine differences in behaviors that might aid in our understanding of their circadian activities.

Arena bioassays were used to determine the bed bug's ability to locate a host from different distances. Starved bed bugs were placed 25, 50, or 100 cm away from a host. The distanced traveled, time to host location, searching speed, and the straightness of the bed bug's path were recorded. As expected, the time to location, distance traveled, and the deviation from the direct path to the host, all increased with an increase in distance. However, all bed bugs were able to successfully locate a host from all three distances. Even bed bugs that had been completely antennectomized were able to successfully locate the host 100% of the time. However, the time to location, path deviation and distance traveled were significantly greater than those same variables recorded for bed bugs with their antennae intact.

Fed and unfed bed bugs were tested both individually and in groups to determine their attraction to aggregation cues. In all tests, bed bugs were attracted to fecal material from conspecifics and fifth instar exuvia. Interestingly, the presence of a live conspecific (either male or female) was not an attractive aggregation cue except to males, regardless of feeding status, in the individual assays. This would suggest that both male and female bed bugs are attractive to other bed bugs, but female bed bugs are not to respond towards conspecifics. Further research into the chemical ecology of bed bug feces and exuvia could have useful application as traps and monitors for both fed and unfed bed bugs in an infestation.

Finally, fed and unfed bed bugs were observed to determine their circadian activities after taking or attempting to take a blood meal. Bed bugs that were able to feed successfully were observed to return to their harborages and aggregate within 30 minutes after feeding. The majority of the fed bed bugs remained in the harborage the entire scotophase. The hungry bed bugs, however, did not aggregate but continued to move around the bioassay arena until approximately two hours before the start of the photophase, at which time they began to return to the harborage and aggregate.

Although bed bugs are cryptic and are becoming increasingly difficult to control with insecticides, the increased knowledge of the bed bug's basic behavior offered by this study provides many possible applications for bed bug control. We have found that bed bugs, both with their antennae intact and fully removed, are very good at locating a host. We have also found that bed bugs, regardless of sex or feeding status, are attracted to feces and exuvia. In addition, recently fed bed bugs are quickly returning to shelters containing feces and other bed bugs. There are many potential integrated pest management strategies that could be derived from this new knowledge.

Chapter 7. References Cited

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