AGGREGATION AND DISPERAL BEHAVIOR OF THE COMMON BED BUG, *Cimex lectularius* L., AND A METHOD OF DETECTION USING CANINES

By

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To my parents
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AGGREGATION AND DISPERsal BEHAVIOR OF THE COMMON BED BUG, *Cimex lectularius* L., AND A METHOD OF DETECTION USING CANINES

By

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Major: Entomology and Nematology

The common bed bug occurs in aggregations that include all life stages but dispersal from these aggregations has not been previously studied. Bed bug aggregation/dispersal behavior was tested in glass Petri dish arenas. The percentages of aggregated and lone bed bugs were observed, as well as the number of aggregations and the number of bed bugs within aggregations. Nymphs had a high tendency to aggregate, varying between 94 to 98%, with an average of two aggregations consisting of 36 to 68 bed bugs. Increasing density did not significantly affect bed bug nymphs. Nymphs were probably not the dispersal stage of the common bed bug because they were not observed being away from aggregations. As the proportion of male bed bugs in arenas increased from 20 to 100%, approximately 21 to 37% males and 26 to 55% females were found alone, which was a significant increase. The number of aggregations was about 2 consisting of approximately 3 bed bugs and was not affected by sex-ratio. At sex-ratio proportions of 20, 50, and 80% males, there were significantly more lone females than lone males. Males left aggregations if the proportion of males was high and there were few females. Because females can be harmed by multiple traumatic inseminations, females may disperse in order to avoid multiple matings with males. As bed bug density increased from
10 to 40, the percentage of aggregated bed bugs significantly increased from 52 to 80%, and the percentage of lone females significantly decreased from 68 to 27%. The percentage of lone males was not affected by density. The number of aggregations significantly increased from 2 to 7, consisting of ~4 bed bugs. The increase in percentage of bed bugs that aggregated as density increased occurred in the female bed bugs. As bed bug density increased, females had a greater tendency to aggregate in female-biased aggregations and were found alone less often. Female bed bugs dispersed from the aggregations, possibly to avoid multiple traumatic inseminations.

The common bed bug is difficult to visually locate because of the many possible harborages where aggregations can occur. Detector dogs were expected to be useful for locating bed bugs because they use olfaction rather than vision. Dogs were trained to detect the common bed bug (as few as one adult male or female) and viable bed bug eggs (5, collected 5-6 days after feeding) using a modified food and verbal reward system. Their efficacy was tested with bed bugs and viable bed bug eggs placed in vented PVC containers. Dogs were able to discriminate bed bugs from *Camponotus floridanus* Buckley, *Blatella germanica* L., and *Reticulitermes flavipes* Kollar, with a 97.5% positive indication rate (correct indication of bed bugs when present) and 0% false positives (incorrect indication of bed bugs when not present). Dogs were also able to discriminate live bed bugs and viable bed bug eggs from dead bed bugs, cast skins, and feces, with a 95% positive indication rate and a 3% false positive rate on bed bug feces. In a controlled experiment in hotel rooms, dogs were 98% accurate in locating live bed bugs. A pseudoscent prepared from pentane extraction of bed bugs was recognized by trained dogs as bed bug scent (100% indication). The pseudoscent could be used to facilitate detector dog training and quality assurance programs. If trained properly, dogs can be used effectively to locate live bed bugs and viable bed bug eggs.
Insects of the family Cimicidae probably evolved as ectoparasites of cave-dwelling mammals. When humans began to inhabit those same caves, some bed bugs probably changed hosts and began infesting humans (Usinger 1966). The common bed bug has probably been associated with humans ever since. References to bed bugs occur in much literature throughout history, occurring in ancient Egyptian writings as far back as 3rd-century B.C. (Strouhal 1995, Panagiotakopulu and Buckland 1999). Aristophane and Aristotle also immortalized this pest in their plays that date back to 423 B.C. (Usinger 1966). One of the oldest pest control companies can be traced back to 1695, when records show the number one pest of that time was the bed bug (Kramer 2004). Although bed bugs practically disappeared from developed countries for about fifty years, they have once again emerged as a leading pest (Kruger 2000). The common bed bug has always been a foe of man, and today is no different.

Bed bugs feed by sucking the blood of humans and other mammals, especially when the hosts are sleeping and therefore unaware that they are being fed upon (Usinger 1966). When associated with humans, bed bugs infest dwellings such as houses, hotels, dormitories, and cruise ships (Doggett et al. 2004). Infestations in human dwellings not only can cause allergic reactions from the bites, but can also cause emotional distress to those affected (Usinger 1966). Therefore control and elimination of this pest is necessary. However, the cryptic nature of the common bed bug makes it difficult to detect and locate (Usinger 1966, Pinto et al. 2007), making control difficult.

Because bed bugs have not been proven to transmit any diseases, interest in learning about this species declined with their declining populations in developed countries. For about fifty years, little new information was discovered about them. When the common bed bug suddenly
reemerged in developed countries in the late 1990s, the lack of knowledge about this insect became apparent. The Monograph of Cimicidae by R. L. Usinger forms the foundation of our knowledge about bed bugs, but this book consists mainly of the author’s own observations or the observations of others, and, in some cases, lacks scientific experimentation. Behavior of the common bed bug is difficult to study in field situations because of their close interaction with humans. As with many pest insects, understanding of the behavior of the pest usually leads to better control methods.

My research focused on two aspects of the common bed bug that are necessary for control to be achieved. The first part focused on bed bug behavior. Bed bugs are found in aggregations (Usinger 1966) but also disperse to other rooms or buildings, either by active walking or hitchhiking on people’s belongings (Pinto et al. 2007). This dispersal behavior facilitates the spread of infestations from one place to another. However, the reasons why bed bugs disperse have not been explored.

The second part of my research focused on detection of the common bed bug. Infestations occur in many places that have a rapid turnover of people, such as hotels and cruise ships (Doggett et al. 2004). Many of the owners/managers of these establishments do not realize they have infested rooms until a customer is bitten and complains, possibly leading to lawsuits (Doggett et al. 2004). Currently, there is no bed bug monitoring device, which would be beneficial to these establishments and perhaps avoid most customer complaints. I evaluated the possibility of training canines to detect the common bed bug. Therefore my thesis explored the causes of bed bug dispersal and detection of bed bugs using canines.
CHAPTER 2  
LITERATURE REVIEW: THE COMMON BED BUG  

Classification and Taxonomy  

Bed bugs are true bugs that are members of the order Hemiptera and the suborder Heteroptera. Insects in this order have unique sucking-piercing mouthparts termed beaks, consisting of four penetrating stylets encircled by a flexible, segmented covering (Usinger 1966, Triplehorn and Johnson 2005). Most Hemipterans feed on fluids from plants, but there are some that have evolved to feed in a similar manner on other insects as well as the blood of birds and mammals. The two main families that have evolved this ability are Reduviidae which include the assassin bugs, wheel bugs, and ambush bugs, and Cimicidae which include the bed bugs, bat bugs, and bird bugs (Boase 2001, Triplehorn and Johnson 2005). There are 20 genera consisting of 74 described species of Cimicidae (Usinger 1966). There are 31 species within the subfamily Cimicinae, and 16 species in the genera *Cimex*, including the species *Cimex lectularius* L., the common bed bug (Usinger 1966). The common bed bug is an ectoparasite of humans but can also feed on other animals such as birds and bats (Usinger 1966, Reinhardt and Siva-Jothy 2007).

The scientific name for the common bed bug is derived from the Roman word for bug, *Cimex*, and the Latin word for small bed, *lectulus* (Ryckman 1979). *C. lectularius*’s beak has three segments and its antennae have four segments (Usinger 1966, Triplehorn and Johnson 2005). They have claws at the end of their forelegs that aid in feeding (Usinger 1966). The common bed bug is very similar in appearance to the tropical bed bug, *Cimex hemipterus* Fabricius, which is associated with humans in tropical climates (Usinger 1966, Pinto et al. 2007).
Adults

The adults of the common bed bug are ~ 5 to 6 mm in length. They are usually a brown to a reddish-brown in color. They are oval in shape and flattened dorsal ventrally, enabling them to hide in small cracks and crevices (Usinger 1966, Krinsky 2002, Harlan and Potter 2004, Triplehorn and Johnson 2005). They have reduced front wing pads and lack rear wings, and so are unable to fly. The reduced fore wings, or hemelytra, are broader than they are long, with a somewhat rectangular appearance (Usinger 1966). The sides of the canoe-shaped pronotum are covered with short, firm hairs (Usinger 1966, Krinsky 2002). The head is cylindrically shaped with two multifaceted eyes and no ocelli (Usinger 1966, Krinsky 2002). *C. lectularius* can easily be distinguished by *C. hemipterus*, the tropical bed bug, by the shape of the pronotum. The pronotum of the common bed bug is more expanded laterally and the extreme margins are more flattened than that of the tropical bed bug (Usinger 1966, Smith 1973, Krinsky 2002).

Males

Males have a narrower abdomen and a longer last segment than females (Usinger 1966, Krinsky 2002). At the posterior tip of the abdomen is a reproductive structure called a paramere. The paramere is a spear-like structure that males use to pierce the female’s abdomen and inject sperm. It is always curved to the left, due to their mating behavior (see below) (Usinger 1966).

Females

Females have a broader, more rounded abdomen than males (Usinger 1966, Pinto et al. 2007). They have a structure called a spermalege that is located on the right ventral side on the fifth abdominal segment (Usinger 1966). This structure is where the male
inserts his paramere to inseminate the female during mating (Usinger 1966, Stutt and Siva-Jothy 2001).

**Nymphs**

Nymphs can range in size from ~1 mm to ~6 mm. Their appearance is similar to that of the adults except they are smaller and they do not have reproductive organs or wingpads. The nymphs are a tan, translucent color. If they have recently had a blood meal, they appear a bright red in color (Usinger 1966, Pinto et al. 2007).

**Eggs**

The eggs are ~1 mm in length and are a pearly white color (Usinger 1966). They have an oval, curved shape (Pinto et al. 2007). A sealed cap, called an operculum, is located at the tip of the egg (Usinger 1966).

**History**

It is hypothesized that bed bugs became associated with humans when humans lived in caves. When humans moved from caves to villages, bed bugs came as well (Usinger 1966, Krinsky 2002, Pinto et al. 2007). Factually, archaeological evidence shows that the common bed bug has been disturbing the sleep of humans for at least the past 3500 years (Panagiotakopulu and Buckland 1999). Bed bugs have also made many appearances throughout documented history, as far back as 3rd-century B.C. from the ancient Egyptians (Strouhal 1995, Panagiotakopulu and Buckland 1999, Pinto et al. 2007). A few other early documentations include Aristophanes’s The Clouds from 423 B.C., and Aristotle’s *Historia Animalium* from somewhere between 384-322 B.C. (Usinger 1966, Krinsky 2002, Pinto et al. 2007). In the mid-eighteenth century in Scotland, only the houses that relied on coal for heat had bed bugs. Coal was a commodity of the rich, so it was typical of the upper society to be infested
(Panagiotakopulu and Buckland 1999, Gangloff-Kaufman and Schultz 2003), which is opposite of the current stigma of the common bed bug.

In more recent history, the common bed bug was prevalent in developed countries up until the end of WWII. The decline of the common bed bug after the war was caused by many reasons. These include improvements in cleaning appliances, novel house designs, greater pest awareness, and broad use of synthetic insecticides such as DDT (Kruger 2000, Boase 2001, Gangloff-Kaufman and Schultz 2003, Pinto et al. 2007).

The resurgence of the common bed bug in developed countries was detected in the late 1990’s. Many hypotheses have arisen as to how this occurred that include a number of different factors, such as increased world travel, reluctance to use toxic chemicals, and insecticide resistance (Kruger 2000, Boase 2001). The tropical origin hypothesis states that the outbreaks are from increased world travel to and from the tropics. Most countries dealing with the resurgence are having problems with the species *Cimex lectularius*, but the species *Cimex hemipterus* is the species that is established in the tropics (Boase 2001). This seems to negate the tropical origin hypothesis as being the sole factor for the resurgence (Boase 2001). Also, there are studies as early as 1963 that record bed bugs as being resistant to DDT and dieldrin, showing resistance in the common bed bug long before the resurgence (Sharma 1963).

**Distribution**

The common bed bug is cosmopolitan in northern temperate climates (Usinger 1966, Rykman et al. 1981, Pinto et al. 2007). In the United States, they have been found in every state (Pinto et al. 2007). Human dwellings, birds’ nests, and bat caves make the most suitable habitats for bed bugs because they offer warmth, areas to hide, and most importantly, hosts on which to feed (Usinger 1966). Common bed bugs have been found
in many types of buildings such as homes, hotels, cruise ships, hostels, and trains (Doggett et al. 2004, Harlan and Cooper 2004).

**Life Cycle**

**Eggs**

When eggs are first laid, they are covered with a wet, sticky substance (Usinger 1966, Harlan and Cooper 2004). As this substance dries, it acts as cement, keeping the egg attached to the surface it was laid on (Usinger 1966). Eggs can be found individually or in clusters close to harborages, with 6-10 being laid per female each week (Usinger 1966, Pinto et al 2007). They hatch in 5-12 days, depending on the temperature (Johnson 1942). At the time of hatching, the *C. lectularius* forces its way through the operculum, which is a sealed cap on the top of the egg. The insect swallows air in order to make itself larger, forcing it out of the egg capsule (Usinger 1966).

Growth and development from hatching to an adult usually takes between 1 and 2 months (Usinger 1966, Pinto et al. 2007). At 18° C, growth and development takes ~128 days; at 30° C, growth and development only takes ~24 days (Johnson 1942, Usinger 1966). The low threshold for egg hatching is between 13 and 15° C, but bed bugs can endure temperatures as low as -15° C for short periods of time (Johnson 1942, Usinger 1966, Pinto et al. 2007). The high threshold for survival is between 44 and 45° C, at which death occurs (Usinger 1966). More recent studies have shown that *Cimex lectularius* will remain active and living if held at a constant temperature as low as 6.6° C (Pinto et al. 2007). Relative humidity has little effect on eggs (Johnson 1942).
Nymphs

Temperature and relative humidity

Growth and development of nympha stages is affected by temperature in the same way as eggs. Relative humidity does not seem to have much affect on common bed bugs until the extremes are reached (Usinger 1966, Pinto et al. 2007). A relative humidity of 20% or less caused death due to desiccation; high relative humidity values caused the growth of fungus, resulting in death, although there is no record of what type of fungus this was (Usinger 1966, Pinto et al. 2007).

Feeding behavior

A bed bug nymph about to feed approaches its host with its antennae and beak extended (Usinger 1966). The tarsal claws of the front legs are critical for gripping the surface and creating the leverage needed to shove the mouthparts through the host’s skin (Pinto et al. 2007). Common bed bugs whose front legs have been amputated cannot feed (Usinger 1966). As the beak pierces the skin of a host, the labium folds back, allowing the stylet to enter more deeply into the host’s skin (Krinsky 2002). The stylet is repeatedly withdrawn and probed into the host (Usinger 1966) until the mouthparts puncture a suitable blood vessel (Lavoipierre 1965).

A newly emerged nymph can feed within the first 24 hours of its life (Usinger 1966). A nymph must get a complete blood meal before it will molt to the next instar. The nymphs of the common bed bug will consume anywhere between 3-6 times their own weight in blood, varying from 0.34 mg to 7.09 mg (Usinger 1966). Nymphs go through 5 instars, thus 5 molts, before reaching the adult stage (Usinger 1966, Harlan and Cooper 2004). Temperature, humidity, and availability of blood meals determine how long it will take a newly hatched nymph to reach adulthood (Usinger 1966).
Salivary components

Most blood sucking arthropods have salivary components capable of inhibiting reactions in their host’s blood (Ribeiro and Francischetti 2003). Common bed bugs have an apyrase in their saliva that cleaves adenosine diphosphate (ADP) to adenosine monophosphate (AMP) (Valenzuela et al. 1996). ADP is necessary for platelet activation and aggregation, so the cleavage of ADP allows the blood of the host to flow continuously (Valenzuela et al. 1996). C. lectularius also has a nitric oxide vasodilator component in its saliva, which keeps blood vessels expanded during feeding (Valenzuela et al. 1995).

Adults

Temperature and relative humidity

Adults are affected by temperature and relative humidity similarly as nymphs and eggs. The lifespan of adults is mostly affected by temperature. At 37° C, females live an average of 32 days while males live an average of 29 days (Usinger 1966). At 10° C, females live an average of 425 days while males live an average of 402 days (Usinger 1966).

Feeding behavior

The feeding behavior of the adults of the common bed bug is similar to that of nymphs, except adults consume more blood. Adult males consume about 1.5 times their weight during feeding (2.37 mg), while females consume about twice their weight (7.81 mg) (Usinger 1966). Adults of the common bed bugs need to feed to acquire the nutrients necessary to provision eggs or develop sperm (Usinger 1966, Stutt and Siva-Jothy 2001). Adults also have the salivary components that the nymphs have.
Mating behavior

Bed bugs copulate by a unique method called traumatic insemination (Usinger 1966, Stutt and Siva-Jothy 2001). During traumatic insemination, the male mounts the female at an oblique angle and actually uses his reproductive organ (paramere) to pierce the body wall of the female’s abdomen (at the spermalege) where he ejaculates into her hemocoel. Insemination occurs completely outside of the female’s reproductive tract (Usinger 1966, Morrow and Arnqvist 2003). Although males will mate with unfed females, their attention is directed to recently fed females (Reinhardt and Siva-Jothy 2007). After sperm is introduced into the female, it migrates from the spermalege through the hemocoel to the ovaries (Usinger 1966, Reinhardt et al. 2003). The details of how this occurs are still unclear (Ribaga 1897, Usinger 1966, Stutt and Siva Jothy 2001, Siva-Jothy and Stutt 2003, Morrow and Arnquist 2003, Reindhardt et al. 2003).

Sexual conflict

There seems to be high sexual conflict between male and female bed bugs. It is beneficial for males to copulate multiple times because traumatic insemination results in last male-sperm precedence (Stutt and Siva Jothy 2001). Males have chemoreceptors on their parameres, which senses the sperm from previous males (Siva-Jothy and Stutt 2003). Males that mate with females who have already mated deposit a smaller amount of sperm in the spermalege than they do when they mate with virgin females (Siva-Jothy and Stutt 2003). Therefore it is more beneficial for males to mate with females that have already mated because they expend less energy and nutrients, but have a higher chance that their sperm will be used to fertilize the female’s eggs (Siva-Jothy and Stutt 2003). However, females only need to mate once every 5-6 weeks to maintain fertility to lay eggs (Usinger 1966). Females copulated an average of 5 times in one week while males
copulated whenever they came in contact with a female (Stutt and Siva-Jothy 2001). In
the same experiment, females that mated 5 times a week had a reduced life and thus
resulted in 24% fewer fertile eggs than females that mated only once every 4 weeks.
Stutt and Siva-Jothy (2001) determined that copulation occurred only during the first 36
hours after a blood meal. This is most likely because fully-engorged females cannot
perform the ‘refusal’ posture and cannot stop the males from mating with them (Siva-
Jothy 2006). Therefore it seems that traumatic insemination is costly to the females.

Although mating is costly to females, the evolution of the spermalege seems to
have arisen in order to reduce the costs of mating (Reinhardt et al. 2003). Reinhardt et al.
(2003) found that the spermalege may protect females from acquiring pathogens during
traumatic insemination. Another study theorizes that the spermalege evolved in order to
localize traumatic piercing to one area of the abdomen, making immune responses more
effective (Morrow and Arnquist 2003).

**Oviposition**

After the initial feeding and mating (and depending on the temperature), it takes the
females approximately six days to lay the first eggs (Usinger 1966). Females prefer to
lay eggs on rough, corrugated surfaces (Usinger 1966). Setae on the legs of *C.
lectularius* may be used to detect surface textures and thus play a role in oviposition site
selection (Walpole 1987). Oviposition lasts for about six days after one feeding, during
which 6-10 eggs are laid. However, oviposition can become continuous if feeding occurs
at least twice a week (Johnson 1942, Usinger 1966, Harlan and Cooper 2004). If they
feed often enough, there is no latent period between egg-laying after the initial six days
(Usinger 1966). Females can lay from 200-500 eggs in their lifetime (Pinto et al. 2007).
Symbionts

Both the endosymbiont *Wolbachia* and a bovine enterovirus-like gamma-proteobacterial symbiont can be found in *Cimex lectularius* (Usinger 1966, Reinhardt and Siva-Jothy 2007). It has been proposed that one of these symbionts, possibly the BEV-like gamma-proteobacteria, provide B vitamins for the common bed bug (Usinger 1966). When symbionts were eliminated by heat (36° C for two weeks) or antibiotics, there was a severe reduction in egg production (Chang 1974, Reinhardt and Siva-Jothy 2007). Although the role of symbionts in the common bed bug is unclear, it seems that they are important to survival (Usinger 1966, Reinhardt and Siva-Jothy 2007).

Disease Transmission

Because bed bugs are obligate blood feeders and every life stage needs to feed, it would seem that bed bugs would be ideal vectors of pathogens (Usinger 1966). Many researchers have hypothesized the common bed bug transmits diseases, but there has been no conclusive demonstration that this occurs (Usinger 1966). A few diseases that bed bugs have been suspected of transmitting are hepatitis, yellow fever, Rocky Mountain spotted fever, leprosy, plague, malaria, and leishmaniasis (Rykman et al. 1981). One study showed that hepatitis B virus could be mechanically transmitted by *C. lectularius*, but there is no evidence that this would occur under normal conditions (Blow et al. 2001).

Although bed bugs have not been shown to transmit any diseases, allergic reactions to common bed bug bites are frequent. The reactions are generally caused by antigens present in the saliva of *C. lectularius* (Samson et al. 1992, Leverkus et al. 2006). A person bitten by a bed bug for the first time should not initially react to the bite unless it cross-reacts with a previous bite from another insect species (Ryckman 1979). There is
a wheal-and-flare response (redness and swelling resulting from the release of histamine) with itching; infiltrated papules, vesicles, or blisters can also develop (Sansom et al. 1992, Liebold et al. 2003). Bites will most likely lead to delayed reactions, with the time between the bite and reaction steadily decreasing with repeated exposure until reactions occur immediately after bites (Usinger 1966, Ryckman 1979). Eventually, one may form immunity to bed bug bites (Ryckman 1979). However, this is not the case in everyone; reactions to bed bug bites vary greatly, from no reaction at all to extreme hypersensitivity (Ryckman 1979).

**Aggregation and Dispersal Behavior**

Bed bugs are found in aggregations (Usinger 1966, Harlan and Cooper 2004, Pinto et al. 2007). These aggregations are all-inclusive; early instars, middle instars, late instars, and both sexes can be found in a single aggregation, as well as bugs of different feeding and mating status (Johnson 1942, Reinhardt and Siva-Jothy 2007). There are many possible reasons that bed bugs aggregate. Within human dwellings, harborages include cracks and crevices in walls, furniture, behind wallpaper, wood paneling and baseboards, or under carpeting (Usinger 1966, Krueger 2000, Pinto et al. 2007).

Aggregations may occur in safe harborages found away from predators (Pinto et al. 2007), as is found in the bug *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) (Lorenzo and Lazzari 1996). Also, solitary bed bugs have a lower resistance to desiccation than aggregated bed bugs do (Benoit et al. 2007), similar to *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Lockwood and Story 1986). It is also possible that aggregations make it easier for adult bed bugs to find mates (Pinto et al. 2007). Aggregation behavior seems to be mediated by pheromones emitted by adults (Reinhardt and Siva-Jothy 2007) as well as nymphs (Siljander et al. 2007). Mechanoreceptors on
bed bug antennae may recognize neighboring bed bugs, possibly contributing to the formation of aggregations (Levinson et al. 1974), because bed bugs are thigmotactic (Usinger 1996).

Although common bed bugs usually aggregate, they disperse by climbing on people’s belongings, and are thus carried to new locations (Usinger 1966, Pinto et al. 2007). They can also actively disperse by actively walking to new locations (Reinhardt and Siva-Jothy 2008). However, the force that drives dispersal in bed bugs is unknown. It could lie in their unique mating behavior. Traumatic insemination seems to cause conflict between male and female bed bugs (Stutt and Siva-Jothy 2001, Siva-Jothy and Stutt 2003, Siva-Jothy 2006, Morrow and Arnquist 2003). It is possible that the female bed bugs are the ones dispersing from the aggregations in order to avoid multiple traumatic inseminations, because it is so costly to the females. However, there are no clear studies testing this theory.

Detection

During the day, bed bugs hide in cracks and crevices that they leave at night in order to feed (Usinger 1966). Because there are so many possible harborages for bed bugs, visual location of the pest can be difficult, but visual detection is the method that is used (Harlan and Cooper 2004). It is especially difficult to locate small, early infestations of bed bugs because of their cryptic, nature (Pinto et al. 2007). To complicate matters many people have delayed reactions to bed bug bites, if any reaction at all (Sansom et al. 1992), making it almost impossible to determine the specific timeframe a person was exposed to an infestation. Also, it is possible that females are dispersing from the aggregations, possibly causing treatment failure. These factors make it difficult to perceive early infestations until the populations are excessive and
overwhelming (Pinto et al. 2007). If infestations are detected early, control is cheaper and more successful (Doggett 2007). Visual identification of a pest is necessary before treatment can occur, causing visual inspections for bed bugs time-consuming and expensive (St. Aubin 1981, Pinto et al. 2007). Therefore, a bed bug-detection method that does not rely solely on visual location would be a vital management tool, especially for detecting early infestations.
CHAPTER 3
AGGREGATION AND DISPERSAL BEHAVIOR OF THE COMMON BED BUG

Introduction

Many nonsocial insects form groups of conspecifics called aggregations. These aggregations are regulated by different types of communication, such as visual, auditory, or chemical (Bradbury and Vehrencamp 1998). The chemical signals that stimulate group organization are called aggregation pheromones, which have been studied in over 300 nonsocial arthropod species including 51 families and 12 different orders (Wertheim et al. 2005). These pheromones can be emitted by any life stage (eggs, nymphs, larvae, pupae) and/or sex, depending on the evolution of the insect (Wertheim et al. 2005). Chemosensory organs, usually located on the antennae, tarsi, or mouth appendages, are used to detect the presence of aggregation pheromones (Borden 1985).

The common bed bug, *Cimex lectularius* (L.) (Hemiptera: Cimicidae) occurs in aggregations (Usinger 1966), consisting of bed bugs of all life stages, feeding status, and mating conditions (Johnson 1942, Reinhardt and Siva-Jothy 2007). Bed bugs may aggregate for multiple reasons. Similarly to *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) (Lorenzo and Lazzari 1996), aggregations may occur in places that are considered safe from predators (Pinto et al. 2007). Also, like *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Lockwood and Story 1986), aggregated bed bugs display a higher resistance to desiccation than solitary bed bugs (Benoit et al. 2007). Aggregations may also assist the bed bugs in finding mates (Pinto et al. 2007). This aggregation behavior seems to be chemically-mediated by pheromones emitted by adults (Reinhardt and Siva-Jothy 2007) as well as nymphs (Siljander et al. 2007). Aggregations may also be initiated by the recognition of neighboring bed bugs by mechanoreceptors on
antennae (Levinson et al. 1974) which may reflect the thigomotactic affinity of bed bugs (Usinger 1966).

The persistence of aggregations depends on a balance between advantages and costs associated with them. There is usually a threshold triggered by different factors that, when reached, causes aggregations to no longer be beneficial to the insects (Wertheim et al. 2005). Aggregated individuals tend to have higher competition for food, space, and mates, may be more apparent to natural predators, can cause deterioration of environmental conditions because of overuse, and can be subjected to inbreeding (Bowler and Benton 2005, Wertheim et al. 2005). These factors can lead to dispersal, or movement away from aggregations, among individuals. For example, the butterfly *Melitaea cinxia* (L.) and the German cockroach *Blattella germanica* (L.) disperse when density is high (Enfjall and Leimar 2005, Ross et al. 1984). The tephritid fly *Paroxyna plantaginis* disperses due to competition for egg-laying substrates, a necessary resource (Albrectsen and Nachman 2001). The pressure to disperse can be unevenly distributed in the population (Bowler and Benton 2005). For example, adults of *M. cinxia* are the dispersal stage (Enfjall and Leimar 2005), whereas middle to late instars of the German cockroach disperse (Ross et al. 1984), and females of *P. plantaginis* are the dispersal stage (Albrectsen and Nachman 2001).

A possible factor driving dispersal in the common bed bug could lie in their reproductive behavior. Bed bugs reproduce by way of traumatic insemination, in which the male uses his spear-like reproductive organ to pierce the female’s abdomen and inject sperm through the wound. Copulation in the common bed bug is known to occur only during the first 36 hours after a blood meal (Stutt and Siva-Jothy 2001). Although females need to mate in order to lay eggs, multiple matings lead to a reduction in life and thus fewer fertile eggs (Stutt and Siva-Jothy...
Males benefit from multiple matings because traumatic insemination seems to favor last male-sperm precedence (Stutt and Siva-Jothy 2001). Because traumatic insemination benefits male bed bugs and elicits costs on female bed bugs, sexual conflict seems to occur (Reinhardt and Siva-Jothy 2007). The possibility of females moving away from aggregations because of their reproductive behavior has not been clearly studied and needs further investigation.

Reports in the literature suggest that females cannot avoid males and thus traumatic insemination associated with males because samples from field populations portray non-biased sex-ratios (Reinhardt and Siva-Jothy 2007). In studies by Johnson (1942), bed bug adults were found to encompass one-third of populations but no sexual bias was observed. In populations collected in KwaZulu (South Africa), adults comprised ~16% of populations and existed at a 1:1 sex-ratio, while nymphs comprised ~84% of populations (Newberry and Jansen 1986). Samples from Dubai and the United Arab Emirates also showed an equal sex-ratio (Stutt and Siva-Jothy 2001). These studies, however, did not look at the composition and behavior of individual aggregations.

The purpose of our study was to determine what factors influence the common bed bug to disperse from aggregations. We tested dispersal as a density-dependent phenomenon, and the possibility of it being related to sex-ratio. We tested the hypothesis that females would leave aggregations and therefore would be found alone more often than males, possibly in avoidance of traumatic insemination, which could possibly improve our understanding of sexual-conflict issues that exist in the common bed bug.

**Materials and Methods**

**Bed Bugs**

The Harlan strain (Harold Harlan, Armed Forces Pest Management Board, U.S. Department of Defense, Washington, DC) of the common bed bug was reared at the University
of Florida’s Department of Entomology and Nematology (Gainesville, FL). The insects were reared as described in Pfiester et al. 2008. In summary, bed bugs were maintained in glass rearing jars lined with filter paper, using pieces of manila folder as harborages. Organdy fabric was placed over the mouth of the rearing jars and secured by a screw-on lid in order to prevent escape. Bed bugs were fed to engorgement weekly on chickens. Bed bugs were harvested with a camel-hair paintbrush when needed, and were fed <2 hours before use in experiments.

**Arena Set-Up**

A square grid (15 x 15 cm) was printed onto filter paper (21.5 x 28 cm, Fisherbrand Qualitative P8). Individual squares within the grid (13 x 13 mm) were labeled by column (vertically, 1-10) and row (horizontally, A-J), corresponding to their location. A circle (14.5 cm diameter) centered at the intersection of squares 6F, 7F, 6G, and 7G, was cut out of the square grid on the filter paper, thus removing from use the individually labeled squares on the corners. The gridded filter paper circle was placed face-up into an inverted Petri dish cover (150 x 20 mm, Pyrex, Corning Incorporated, Corning NY). After addition of bed bugs (see experiments below), the Petri dish bottom was inverted into the Petri dish cover to close the arena and press on the filter paper, preventing the bed bugs from crawling underneath the filter paper. Yellow theatrical gel (~22 x 36 cm, SG/Lux # 10, Rosco Laboratories Inc., Stamford, CT) was placed around the inverted Petri dish and secured to the bottom with scotch tape because it made bed bugs behave as though it was dark. The wrapped Petri dish was then placed on an inverted deli cup where bed bugs were left undisturbed and therefore free to move within the arena. The room the arenas were placed in was used daily, so the light cycle varied throughout the experiment while the temperature remained steady at ~24° C. All experiments were performed in the same room.
Early-Instar Density Experiment

First instar bed bugs were harvested and placed into the center of each arena at varying densities of 50, 100, and 150 bed bugs per arena. Each density was replicated 5 times and data were recorded daily at 4:00 PM for 5 days. The data recorded included the percentage of lone nymphs, the percentage of aggregated nymphs, the number of aggregations, and the number of insects in each aggregation. A lone bed bug was defined as one separated from any other bed bug by a distance greater than the length of one adult bed bug. For all experiments, an aggregation was defined as two or more bed bugs separated by a distance less than or equal to the length of one adult bed bug.

Adult Density Experiment

Adult bed bugs were harvested, sexed, and placed into the center of each arena at varying densities of 10, 20, and 40 individuals per arena, at a 1:1 sex-ratio. Each density was replicated 5 times and data were recorded daily at 4:00 PM for 5 days. The data recorded included the percentage of lone males and lone females, the percentage of aggregated adults, the number of aggregations, and the number and sex of insects in each aggregation.

Life-Stage Experiment

Bed bug nymphs were separated into 3 categories: early-stage (1st or 2nd instar), mid-stage (3rd or 4th instar), and late-stage (5th instar). Adult bed bugs and nymphs of all life-stages (30 total bed bugs per arena) were harvested and placed into the center of each arena at varying compositions of 20, 40, 60, and 80% adults. Adults used were at a 1:1 sex-ratio, and nymph population was at a 1:1:1 life-stage ratio. Each composition was replicated 4 times and data were recorded daily at 4:00 PM for 5 days. The data recorded included the percentage of lone males, lone females, and lone nymphs, the percentage of aggregated bed bugs, the number of aggregations, and the number of insects in each aggregation.
**Sex-Ratio Experiment**

Adult bed bugs were harvested and sexed. Ten adult bed bugs were placed into the center of each arena at varying sex ratios of 100, 80, 50, 20, and 0% males. Each sex-ratio was replicated 9 times and data were recorded daily at 4:00 PM for 5 days. The data recorded included the percentage of lone males and lone females, the percentage of aggregated adults, the number of aggregations, and the number of insects in each aggregation.

**Statistical Analysis**

Percentages were arcsine square root transformed and data were analyzed by analysis of variance with repeated measures (daily observations). Means were separated with Student-Newman Keuls (P < 0.05; SAS Institute, 2003).

**Results**

**Early-Instar Density Experiment**

Released bed bugs proceeded to form aggregations along the edges of the arenas. There were few lone nymphs, ranging from approximately 1-3% (Table 1), and the percent of lone nymphs was not significantly affected by density (df = 2, 12; $F = 3.38; P = 0.0685$). Most of the nymphs were found in aggregations and the percent of aggregated nymphs was also not affected by density (df = 2, 12; $F = 0.89; P = 0.4355$). As density increased, the percent of aggregated nymphs was rather consistent, ranging from 96-98%. Also, the number of aggregations remained steady as density increased, ranging from ~1 to 3 (df = 2, 12; $F = 1.82; P = 0.2044$). Furthermore, the number of insects in each aggregation was not affected by density either (df = 2, 12; $F = 3.34; P = 0.0701$), although the aggregations did grow larger as the density of bed bugs in arenas increased. Aggregations grew from ~36 insects at a density of 50 to ~68 insects at a density of 150.
Adult Density Experiment

As density increased (Table 2), the percent of lone females significantly decreased, from 68% at a density of 10 to ~27% at a density of 40 (df = 2, 12; $F = 12.80; P = 0.0011$). The percent of lone males was not affected by density (df = 2, 12; $F = 2.58; P = 0.1167$). The percent of bed bugs that aggregated significantly increased as density increased, from ~52% to ~80% (df = 2, 12; $F = 17.00; P = 0.0003$), as did the number of aggregations from ~2 to ~7 (df = 2, 12; $F = 103.54; P < 0.0001$). The number of insects in each aggregation significantly increased at a density of 40 (df = 2, 12; $F = 14.19; P = 0.0007$). With 10 adults in the arenas, the percentage of lone females was significantly higher than lone males ($F = 21.03; P = 0.0018$), which also occurred when there were 40 adults in the arenas ($F = 16.23; P = 0.0038$). There was no significant difference between lone males and females when there were 20 adults in the arenas ($F = 1.15; P = 0.3144$). As density of bed bugs increased, significantly more females aggregated in female-biased aggregations than male-biased aggregations. At densities of 20 ($F = 28.50; P = 0.0007$) and 40 ($F = 52.42; P < 0.0001$), significantly more females were found in female-biased aggregations than in male biased aggregations (Fig. 1), but at a density of 10 there was no difference ($F = 4.26; P = 0.0729$).

Population Composition Experiment

Of the bed bugs released in the arenas, there were few lone nymphs in this experiment (Table 3). The percent of lone nymphs (df = 3, 12; $F = 0.36; P = 0.7807$), the percent of lone adult males (df = 3, 12; $F = 0.60; P = 0.6247$), and the percent of lone adult females (df = 3, 12; $F = 1.33; P = 0.3117$) were not affected by the population composition. Increasing the proportion of adults in the arenas did not significantly affect the percent of bed bugs that aggregated (df = 3, 12; $F = 0.76; P = 0.5404$), the number of aggregations (df = 3, 12; $F = 1.32; P = 0.3126$) nor the number of bed bugs per aggregation (df = 3, 12; $F = 0.87; P = 0.4819$).
Male and female adults, however, were found alone at a greater rate than nymphs. The percent of lone nymphs ranged from approximately 3 to 6%, while lone males ranged from about 5 to 15% and the lone females ranged from ~12 to ~20%.

**Sex-Ratio Experiment**

Sex-ratio (Table 4) significantly affected the percent of lone males (df = 3, 32; $F = 3.90; P = 0.0176$) and lone females (df = 3, 32; $F = 6.31; P = 0.0017$). As the proportion of males increased in arenas, the proportion of lone males and lone females also increased. Similarly to the adult density experiment, the percent of lone females was significantly higher than the percent of lone males at sex-ratios of 20 ($F = 16.61; P > 0.0015$), 50 ($F = 20.83; P > 0.0003$), and 80% males ($F = 22.65; P > 0.0002$). The percent of adult bed bugs that aggregated was not significantly affected by sex-ratio (df = 4, 40; $F = 1.61; P = 0.1908$), although females had a greater tendency to aggregate when more females were present. Sex-ratio also had no significant affect on the number of aggregations (df = 4, 40; $F = 0.20; P = 0.9347$) and the number of insects in each aggregation (df = 4, 40; $F = 1.23; P = 0.3148$).

**Discussion**

Our experiments were conducted with a strain of bed bugs that has been lab-reared for at least 30 years. The bed bug strain we used is accustomed to being in close proximity with each other because they are contained in such small jars. Therefore the females are most likely exposed to multiple traumatic inseminations. Female bed bugs in field strains may exhibit more male-avoidance behavior than our lab strain because field female bed bugs may have the opportunity to avoid constant close proximity to males. Also, these experiments reported here were performed in small experimental arenas that did not simulate all the complexity found in field situations. However, observation of bed bug behavior is extremely difficult in field
situations and the necessary control of different factors would not have been possible as it was in a laboratory setting.

Aggregations occur because they provide some benefit for individuals involved that is not present when individuals are solitary. Typically, aggregations may reach a point when they are no longer beneficial, leading to dispersal. Dispersal can be density-dependent and can be driven by a specific life-stage. The common bed bug occurs in aggregations, but can also be found far from main aggregations.

Dispersal of nymphs, as seen in the German cockroach (Ross et al. 1984), does not seem to occur in the common bed bug. In our experiments, the nymphal stages of the common bed bug had a strong tendency to aggregate, with ~94 to ~98% of individuals found in aggregations. Nymphs, which lose water faster when solitary (Benoit et al. 2007), seem to greatly benefit from the aggregation behavior. Also, nymphs produce aggregation pheromone that attracts other nymphs (Siljander et al. 2007). The low numbers of nymphs observed alone in our experiments suggest that the nymphs are not responsible for dispersal in the common bed bug.

Among adults, females had the highest percentage of lone individuals, especially in populations with high proportions of males. Females seem to move away from male-biased aggregations possibly to avoid males and thus traumatic insemination. Although females need to mate in order to lay eggs, aggregations may be detrimental to females due to negative effects of traumatic insemination that reduce the life span of females and the number of fertile eggs (Stutt and Siva-Jothy 2001). Our results showed that adult females moved away from aggregations more often than any other stage, and were likely candidates for dispersal, possibly driven by the avoidance of males.
Although females almost always represented the highest percentage of lone bed bugs, males were found alone quite often. As the proportion of males increased in an arena, the proportion of lone males also increased. This trend can be explained by the need for males to search for females to mate. A previous study determined that males copulated as often as possible in the first 36 hours after feeding (Stutt and Siva-Jothy 2001). Recently fed males may abandon aggregations in search of females. The presence of an aggregation pheromone in males, which arrests or attracts other males and females (Siljander et al. 2007), might negate the need for males to search for females in field populations. However, our results indicate that males may also participate in dispersal in the common bed bug although dispersal of inseminated females would be sufficient to spread populations of this pest.

In our experiments, the percentage of aggregated males did not greatly vary as density of adults increased, but the percent of aggregated females increased with density. However, this increase in aggregation of females occurred mostly in female-biased aggregations. Females may benefit from avoiding males at low densities by leaving aggregations, whereas at high densities, females may avoid males by aggregating with other females. A similar situation is found with the African damselfly, *Platycypha caligata*, where females form groups to avoid courting males and benefit by a higher oviposition rate (Martens and Rehfeldt 1989, Wertheim et al. 2005). Unlike nymphs and males of the common bed bug, females do not produce any aggregation pheromone (Siljander et al. 2007), contrary to previous reports (Usinger 1966, Levinson & Bar Ilan 1971). The lack of aggregation pheromone production can be beneficial to females if they are dispersing from aggregations to avoid males and traumatic insemination. Because females do not release aggregation pheromone (Siljander et al. 2007), the female-biased aggregations we observed may result from the lack of a force driving females to disperse from the aggregations.
This allows females to benefit from female-biased aggregations, for instance by reducing water loss (Benoit et al. 2007) and protecting from multiple traumatic inseminations.

Our results suggest that the common bed bug occurs in dynamic, changing aggregations, unlike the constant aggregations suggested in the literature (Usinger 1966). In our experiments, bed bugs did aggregate, especially nymphs and adult males, but adult females had a high tendency to be alone, away from aggregations, or in female-biased aggregations. The males, in search of females, may leave aggregations when the proportion of males is too high. These results suggest that the composition of aggregations may change over time. This phenomenon cannot be observed when sex-ratio studies are done at the population level, where sex-ratio exists typically at 1:1 (Newberry and Jansen 1986, Stutt and Siva-Jothy 2001, Reinhardt and Siva-Jothy 2007). However, if females that are exposed to multiple traumatic inseminations have a shorter life-span, than a male-biased sex-ratio might have been expected in field populations unless males naturally have a shorter life-span than females.

Because the common bed bug is found to exist in field populations at a 1:1 sex-ratio, it has been suggested that females cannot avoid males and traumatic insemination (Reinhardt and Siva-Jothy 2007). However, if females are actively avoiding males and traumatic inseminations by dispersal behavior as well as by forming female-biased aggregations, samples taken from field populations may show an unbiased sex-ratio, although local aggregations may be female- or male-biased.

The aggregation and dispersal behavior of the females did not change over the 5 days in all our experiments. This seems to be in conflict with previous observations (Stutt and Siva-Jothy 2001) that mating occurs only in the first 36 hours after feeding. If traumatic insemination is only occurring within the first two days, females would only need to avoid males for that time.
period, and a change in aggregation/dispersal behavior would have been expected two days after blood feeding. This change of aggregation/dispersal behavior was not observed in our experiments although we extended observations well beyond 36 h. after blood feeding.

Our results suggest that aggregation and dispersal in the common bed bug may be a dynamic process that may allow females to have an active choice in whether or not they mate. Further observations on mating behavior associated with different aggregation conditions may provide additional information on this phenomenon.
Figure 3-1. Percentage of aggregated females in female- and male-biased aggregations at various densities.
Table 3-1. Effect of density (mean ± SE) on aggregated and lone first-instar nymphal bed bugs (*Cimex lectularius*), the number of aggregations, and the number of insects in aggregations of nymph only populations.

<table>
<thead>
<tr>
<th>Density</th>
<th>% Lone (^a)</th>
<th>% Aggregated (^b)</th>
<th>Number of Aggregations</th>
<th>Number of Insects in Aggregations</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3.1 ± 0.46</td>
<td>95.7 ± 1.07</td>
<td>1.7 ± 0.18</td>
<td>35.8 ± 2.92</td>
</tr>
<tr>
<td>100</td>
<td>0.8 ± 0.17</td>
<td>96.5 ± 2.44</td>
<td>2.5 ± 0.13</td>
<td>43.2 ± 2.84</td>
</tr>
<tr>
<td>150</td>
<td>1.2 ± 0.15</td>
<td>98.1 ± 0.19</td>
<td>2.6 ± 0.20</td>
<td>67.7 ± 6.59</td>
</tr>
</tbody>
</table>

There were no significant differences for all variables analyzed by analysis of variance with repeated measures (P=0.05; Student Newman-Keuls; SAS Institute 2003).

\(^a\)A lone bed bug was defined as a one separated from any other bed bug by a distance greater than the length of one adult bed bug.

\(^b\)An aggregation was defined as two or more bed bugs separated by a distance less than or equal to the length of one adult bed bug. Average mortality was less than 3 %.
Table 3-2. Effect of density (mean ± SE) on aggregated and lone adult bed bugs (Cimex lectularius), the number of aggregations, and the number of insects in aggregations of adult only populations with a 1:1 sex-ratio.

<table>
<thead>
<tr>
<th>Density</th>
<th>% Lone Females&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Lone Males&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Aggregated&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Number of Aggregations</th>
<th>Number of Insects in Aggregations</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>68.0 ± 4.76&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>27.2 ± 4.14&lt;sup&gt;B&lt;/sup&gt;</td>
<td>52.4 ± 3.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>39.2 ± 4.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.2 ± 4.20</td>
<td>65.8 ± 3.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.3 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>26.8 ± 1.47&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>14.2 ± 1.91&lt;sup&gt;B&lt;/sup&gt;</td>
<td>79.5 ± 1.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.4 ± 0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.4 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means in a column followed by the same lowercase letter are not significantly different when analyzed by analysis of variance with repeated measures (P=0.05; Student-Newman-Keuls; SAS Institute 2003).

<sup>a</sup>Lone male and female columns were compared at each density level and significance was indicated by capital letters.

<sup>b</sup>A lone bed bug was defined as one separated from any other bed bug by a distance greater than the length of one adult bed bug.

<sup>b</sup>An aggregation was defined as two or more bed bugs separated by a distance less than or equal to the length of one adult bed bug.
Table 3-3. Effect of population composition (mean ± SE) on aggregated and lone adult and nympha bed bugs (*Cimex lectularius*), the number of aggregations, and the number of insects in aggregations of mixed populations with nympha and adult bed bugs.

<table>
<thead>
<tr>
<th>Population Composition (% Adults)</th>
<th>% Lone Nymphs&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Lone Males&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Lone Females&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Aggregated&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Number of Aggregations</th>
<th>Number of Insects in Aggregations</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>5.6 ± 1.10</td>
<td>15.0 ± 4.51</td>
<td>11.7 ± 3.65</td>
<td>91.5 ± 1.33</td>
<td>3.2 ± 0.25</td>
<td>9.5 ± 1.14</td>
</tr>
<tr>
<td>40</td>
<td>3.0 ± 0.62</td>
<td>10.8 ± 2.78</td>
<td>19.2 ± 2.78</td>
<td>91.0 ± 1.08</td>
<td>3.5 ± 0.31</td>
<td>9.6 ± 1.24</td>
</tr>
<tr>
<td>60</td>
<td>5.7 ± 2.57</td>
<td>12.2 ± 3.21</td>
<td>19.4 ± 3.94</td>
<td>88.2 ± 2.20</td>
<td>4.3 ± 0.36</td>
<td>7.5 ± 0.90</td>
</tr>
<tr>
<td>80</td>
<td>5.0 ± 1.75</td>
<td>5.0 ± 1.64</td>
<td>19.6 ± 2.85</td>
<td>87.3 ± 1.07</td>
<td>4.5 ± 0.34</td>
<td>6.7 ± 0.62</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adult bed bugs and nymphs of all life-stages were used at varying compositions of 20, 40, 60, and 80% adults. Adults used were at a 1:1 sex-ratio, and nympha population was at a 1:1:1 life-stage ratio; early-stage (1<sup>st</sup> or 2<sup>nd</sup> instar), mid-stage (3<sup>rd</sup> or 4<sup>th</sup> instar), and late-stage (5<sup>th</sup> instar).

<sup>b</sup>A lone bed bug was defined as one separated from any other bed bug by a distance greater than the length of one adult bed bug.

<sup>c</sup>An aggregation was defined as two or more bed bugs separated by a distance less than or equal to the length of one adult bed bug.

There were no significant differences at all variables when analyzed by analysis of variance with repeated measures (P=0.05; Student-Newman-Keuls; SAS Institute 2003).
Table 3-4. Effect of sex-ratio (mean ± SE) on aggregated and lone adult bed bugs (*Cimex lectularius*), the number of aggregations, and the number of insects in aggregations of adult only populations with varying sex-ratios.

<table>
<thead>
<tr>
<th>Sex-Ratio (% Males)</th>
<th>% Lone Males</th>
<th>% Lone Females</th>
<th>% Aggregated</th>
<th>Number of Aggregations</th>
<th>Number of Insects in Aggregations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>25.9 ± 2.31a</td>
<td>74.1 ± 2.31</td>
<td>2.2 ± 0.10</td>
<td>3.8 ± 0.25</td>
</tr>
<tr>
<td>20</td>
<td>21.3 ± 4.87aA</td>
<td>38.9 ± 3.22abB</td>
<td>64.6 ± 2.96</td>
<td>2.1 ± 0.13</td>
<td>3.6 ± 0.22</td>
</tr>
<tr>
<td>50</td>
<td>20.7 ± 2.64abA</td>
<td>50.4 ± 3.20bB</td>
<td>64.4 ± 2.16</td>
<td>2.1 ± 0.10</td>
<td>3.5 ± 0.20</td>
</tr>
<tr>
<td>80</td>
<td>35.4 ± 3.28bA</td>
<td>54.6 ± 4.99bB</td>
<td>60.7 ± 2.95</td>
<td>2.1 ± 0.11</td>
<td>3.0 ± 0.23</td>
</tr>
<tr>
<td>100</td>
<td>37.4 ± 3.17b</td>
<td>-</td>
<td>62.6 ± 3.17</td>
<td>2.2 ± 0.10</td>
<td>2.8 ± 0.18</td>
</tr>
</tbody>
</table>

Means in a column followed by a different lowercase letter are significantly different when analyzed by repeated measures analysis of variance (P=0.05; Student-Newman-Keuls; SAS Institute 2003).

*Lone male and female columns were compared at each density level and significance was indicated by capital letter
*aA lone bed bug was defined as one separated from any other bed bug by a distance greater than the length of one adult bed bug.
*bAn aggregation was defined as two or more bed bugs separated by a distance less than or equal to the length of one adult bed bug.
CHAPTER 4
ABILITY OF BED BUG DETECTING CANINES TO LOCATE LIVE COMMON BED BUGS AND VIABLE BED BUG EGGS

Introduction

Archaeological evidence shows that the obligate hematophagous common bed bug, *Cimex lectularius* L., has been disrupting the sleep of humans for at least the past 3,500 years (Panagiotakopulu and Buckland 1999). The decline of bed bug numbers in developed countries after the end of World War II was caused by multiple factors such as novel house designs, improvements in cleaning appliances, and the widespread use of synthetic insecticides such as DDT (Gangloff-Kaufman and Schultz 2003, Kruger 2000). The resurgence of common bed bugs in the developed world was detected in the late 1990s, and calls to pest control professionals for bed bug infestations have increased as much as 4,500% in Australia and 4,783% for one pest control company in the United States (Doggett and Russell 2007, Black 2007).

Bed bugs hide in cracks and crevices during the day where they remain unseen, and come out during the night to feed (Usinger 1966). The variety of bed bug harborages makes visual detection challenging (Cooper and Harlan 2004). Their cryptic nature especially makes it difficult to discover small, early infestations (Pinto et al. 2007). Because many pest control operators will not apply insecticide if they cannot visually locate the pest, inspections are essential but can be time-consuming (St. Aubin 1981). Also, many people have delayed reactions to bed bug bites or even no reaction at all (Sansom et al. 1992), making it difficult to correlate reactions with a specific timeframe a person could have been exposed to an infestation. The difficulties of confirming bed bug infestations cause most early infestations to go unnoticed until the populations are overwhelming (Pinto et al. 2007). Early control of infestations is more likely to succeed, and these infestations are less likely to spread and are cheaper to control.
(Doggett 2007). Therefore, a method that complements visual location of bed bugs would be valuable in live bed bug detection, especially for small and early infestations.

Dogs rely on olfaction rather than vision and have been used to detect a wide variety of materials, such as gases odorless to humans (Johnson 1977), black-footed ferrets (Reindl-Thompson et al. 2006), brown tree snakes (Engeman et al. 1998), explosives, and even missing people (Ashton and Eayrs 1970). There are also accounts of dogs trained to locate insects, such as gypsy moths (Wallner and Ellis 1976), screwworm pupae and larvae (Welch 1990), and termites (Brooks et al. 2003). Bed bug-detecting canines are currently being used at least in the United States and Australia (Cooper 2007, Doggett 2007). The quality of bed bug detecting canines depends on the efficiency of their training and what the dogs are trained to do (Cooper 2007). A high accuracy for bed bug dogs is essential because people want bed bugs to be eliminated, not just a reduction in population (Pinto et al. 2007).

In order for bed bug detecting canines to achieve a high level of accuracy, they should be able to differentiate bed bugs from other cryptic pests and environmental factors commonly found in the same location, such as ants, cockroaches, termites, and mold. Also, they should be able to differentiate live bed bugs and viable eggs from bed bug debris (feces, cast skins, and dead bed bugs) because the presence of bed bug debris does not necessarily indicate a live infestation (Pinto et al. 2007). Therefore, bed bug-detecting dogs are usually trained using target odors (live bed bugs and viable eggs) that are separated from nontarget odors (other general household pests, bed bug debris). However, as bed bugs defecate and shed their skins inside training apparatuses, nontarget odors (debris) must be removed or the dogs would be inadvertently trained to respond to them (United States Customs Service 1979). For example, a dog that was trained on both termites and wood debris had a false positive indication rate of
almost 75%, meaning the dog indicated the presence of termites when only termite damaged wood was present (Brooks et al. 2003). To simplify training, a termite pseudoscent was developed for trainers and handlers of termite-detecting canines, reducing the possibility of training dogs on nontarget odors (Brooks 2001).

The purpose of our study was to determine the ability of canines to detect common bed bugs when trained with live adult bed bugs. The first objective was to determine if trained dogs are able to differentiate bed bugs from other general household pests, such as Florida carpenter ants, *Camponotus floridanus* Buckley, German cockroaches, *Blatella germanica* L., and eastern subterranean termites, *Reticulitermes flavipes* Kollar. Secondly, we wanted to determine if dogs could be trained to discriminate live bed bugs and viable eggs from other bed bug materials, such as fecal deposits, cast skins, and dead bed bugs. We also wanted to verify that, in a controlled experiment, trained dogs could locate hidden bed bugs in hotel rooms. Finally, we wanted to test different solvent extractions to see if a bed bug pseudoscent could be recognized as live bed bugs by trained dogs.

**Materials and Methods**

**Bed Bugs**

The Harlan strain (Harold Harlan, Armed Forces Pest Management Board, U.S. Department of Defense, Washington DC) of the common bed bug was reared at the University of Florida’s Department of Entomology and Nematology (Gainesville, FL). The insects were maintained in 240-ml glass rearing jars (Ball Collection Elite, Jarden Home Brands, Muncie, IN) with a 90-mm filter paper circle (Whatman #1) on the bottom of the rearing jar. Harborages were made from rectangles of manila folder (90 mm x 60 mm) folded in a fan-like manner and placed inside each jar.
Bed bugs were separated with feather-tipped forceps and placed into rearing jars according to life stage (~200 bed bugs in each jar). As adults laid eggs, the eggs were placed into new rearing jars weekly. This was done by placing the rearing jar on ice to knock down the adults and transferring the filter paper and harborage with the eggs attached into a new rearing jar. New paper and harborage was added to the rearing jar containing the adults. To prevent insect escape, organdy fabric was placed over the mouth of the rearing jar and secured by a screw-on lid. Bed bugs were maintained at 23-24 °C with a relative humidity of ~50% and a photoperiod of 12:12 (L:D).

Bed bugs were fed to engorgement once a wk on chickens (IACUC protocol # E876). The chickens were bound at the feet and hooded, and the feathers on the side of the chickens’ breasts were shaved to expose skin. The rearing jars of bed bugs were placed upside down on the shaved skin and the bed bugs fed through the organdy cloth. Bed bugs were harvested with a camel-hair paintbrush ~2 hr before working with the dogs.

**General Household Pests**

Orlando strain German cockroaches were reared in large glass utility jars containing cardboard harborages. Dry food (23% crude protein: PMI Nutrition International, Inc. Lab Diet 5001 rodent Diet, Brentwood, MO) and water were provided *ad libitum*. The cockroaches were maintained at 23-24 °C with a relative humidity of ~50% and a photoperiod of 12:12 (L:D).

Eastern subterranean termites were collected from a single colony (Gainesville, FL). They were given damp cardboard and maintained at 23°C with a relative humidity of 55% and a photoperiod of 12:12 (L:D).

Florida carpenter ants were reared at the USDA -ARS laboratory in Gainesville, FL, at a temperature range of 26-28 °C. They were fed crickets five days a wk, hard boiled eggs once a
wk, and given 10% sugar water and water *ad libitum*. All general household pests were handled with feather-tipped forceps to prevent damage to the insects.

**General Household Pests, Bed Bug Debris, and Hotel Field Experiment Scent Vials**

Filter paper (90 mm x 40 mm) was folded in a fan-like manner and placed in a plastic snap-cap vial (18.5 mL, Thornton Plastic Co., Salt Lake City, UT). A hole (~15 mm diameter) was cut into the cap. Organdy fabric (60 mm x 60 mm) was placed over the vial opening and held in place with the cap. Multiple vials were prepared and five of either live adult bed bugs (mixed sexes), carpenter ants, termites, cockroaches, viable bed bug eggs, dead adult bed bugs, or bed bug cast skins were placed in the vials. For the hotel field experiment, six scent vials were prepared containing one, five, or ten male-only or female-only adult bed bugs. Vials were also prepared with filter paper that was taken from the rearing jars and contained bed bug feces deposits of various ages. Control vials were prepared with only filter paper inside them. All scent vials were used within 2 h of preparation.

**Pseudoscent Extracts and Scent Vials**

Fifty live, mixed sex, adult bed bugs were placed in each of 4 glass vials (15 ml, Fisher Scientific Co., Pittsburgh, PA). Ten ml of either pentane, methanol, acetone, or water were added to the vials. Vials with solvent and bed bugs were swirled for 10 min. Solvents were then pipetted out of the vials and placed into separate clean glass vials. Vials containing the different solvent extractions were then sealed until use later the same day.

Snap-cap vials with filter paper and organdy fabric were prepared as in the general household pest and bed bug debris experiments. Fifteen min before the experiment, 1 ml of the extract (equivalent to 5 bed bugs) was placed on the filter paper inside separate snap-cap vials. A snap-cap vial containing only filter paper was used as a control. It was previously determined that dogs do not indicate on pentane, methanol, acetone, or water.
**Scent-Detection Stations**

A scent-detecting station consisted of a capped PVC pipe (50 mm diameter x 150 mm height) that was secured onto a recycled plastic board (17 x 48 x 4 cm). A hole (30 mm diameter) was drilled into the center of the PVC cap to allow scent to escape the station after scent vials were placed inside the PVC tube and on top of the plastic board, ~10 cm from the opening of the PVC tube.

**Canines**

Seven dogs were used in the following experiments (IACUC protocol # E732). Dog A was a 10 yr old spayed female Beagle. Dog B was a 4 yr old spayed female Chinese Crested. Dog C was a 2 yr old spayed female Beagle mix. Dog D was a 2 yr old spayed female Beagle mix. Dog E was a 1 yr old neutered male Jack Russel Terrier. Dog F was a 1 yr old spayed female Beagle. Dog G was a 2 yr old neutered male Beagle.

**Canine Training Method**

Scent vials containing live bed bugs and viable bed bug eggs were prepared as above and were placed in scent-detection stations. Dogs were trained to scratch at a scent-detection station containing either the live bed bugs or viable eggs by a modified food and verbal reward method (Brooks et al. 2003). During training, other scent vials containing distracting substances (eg. dog food, human scent, German cockroaches, and bed bug cast skins) were placed in stations to ensure that the canines were alerting only to the odor of the live bed bugs or viable bed bug eggs. Once the bed bug scent was associated with the reward, the canines were fed only after they indicated on the scent of live bed bugs or viable bed bug eggs. All dogs went through 90 d of initial training before being used in the experiments. After the initial training was completed, dogs were maintained by feeding them twice daily only after locating the target odor. In order
to ensure optimal performance, individual dogs were never worked in any experiment for more
than 40 min a day (Brooks et al. 2003).

General Household Pest Experiment

Five scent-detection stations were used in this experiment, each containing a scent-
detection vial of either live bed bugs, cockroaches, termites, ants, or a control vial. Vials were
placed inside the scent-detection stations. Scent-detection vial contents were written on the PVC
cap with invisible ink that could only be seen using an ultraviolet light. This was done to prevent
the dog-handler from knowing which insect was in the station. All stations were marked with
invisible ink to prevent the dogs from detecting the presence of the ink.

The five stations were placed in a line ~1 m apart from each other. The dog-handler
walked the dog down the line, allowing the dog to sniff each station. If the dog missed a station,
the handler was allowed to turn the dog around and walk it past the station again. If the dog did
not indicate on any station, the dog and handler were allowed to walk down the line of stations a
second time. The order of the stations was chosen randomly for each repetition. In total, four
dogs (A, B, C, D) using one handler were evaluated with 20 repetitions each. The data were
taken over a 10 mo period.

As the dogs were evaluated, one of three outcomes was recorded depending on the
performance of the dog; a positive indication, a false positive, or no indication. If the handler
interpreted an indication by the dog at a station, the handler checked with the evaluator to
determine if bed bugs were present. If bed bugs were present, the indication was scored as a
positive indication, and the dog was rewarded. If bed bugs were not present, the indication was
scored as a false positive, and the dog was not rewarded. If the handler did not interpret an
indication by the dog at any station, it was recorded as no indication.
**Bed Bug Debris Experiment**

Six scent-detection stations were used in this experiment, each one containing a scent-detection vial with five of either bed bug cast skins, dead bed bugs, bed bug feces, viable eggs (collected 5-6 days after adult feeding), live adult, mixed sex bed bugs, or a control vial. The labeling, positioning, and randomization of the stations were completed as previously described in the general household pest experiment. Dog evaluation and scoring procedures were also as described previously, except dogs were rewarded for positive indications on live bed bugs and viable eggs. Three dogs (A, B, D) using one handler were evaluated with 20 repetitions each. The data were taken over a 10 mo period.

**Hotel Room Field Experiment**

Six scent vials were used in this experiment, each containing one, five, or ten male-only or female-only adult bed bugs. Two double queen bed hotel rooms were used, one room containing only scent vials with female bed bugs, and the other room containing only scent vials with male bed bugs. Both hotel rooms were identical in size and had similar furniture with the same pattern of arrangement (Fig. 1). For each repetition, the scent vials were randomly hidden in any of seventeen possible locations in each room; the four corners of bed one, the two corners of the nightstand, the four corners of bed two, the two corners of the arm chair, the desk chair, the two corners inside dresser drawer one, or the two corners inside dresser drawer two. All vials were hidden from view of both the dog and the dog handler. Scent vials hidden in the bed were placed between the mattress and boxspring ~ 5 cm from the edge. In the night stand, scent vials were placed in the inside front corners of the open face. Scent vials hidden in the sitting chair were placed under the cushion ~ 5 cm from the edge. In the desk chair, scent vials were placed in the crevice where the backrest and seat join. All four dresser drawers were opened slightly to allow
the dogs access to the scent. Because of this, scent vials were only placed in the bottom two drawers so the handler would not be able to see them.

The dogs were walked through the rooms following the same path for each repetition. The dog/handler team passed the possible locations of hidden bed bugs in the order stated in the previous paragraph. Dogs were allowed two passes in the room if needed. Scent vials were randomly moved to new locations between each run. Fifteen minutes elapsed between runs to allow the scent at the old locations to dissipate and to allow the scent to accumulate at the new locations. Three dogs (A, B, G) using one handler were evaluated with 6 repetitions each. Data were taken over a one-week period.

**Pseudoscent Extracts Experiment**

Five scent-detection stations were used in this experiment and contained a scent-detection vial of either pentane, acetone, methanol, or water extracts, or a control vial. The labeling, positioning, and randomization of the stations were completed as previously described. Dog evaluation and scoring procedures were also as described previously, except dogs were rewarded for indication on any of the scent-detection stations except the control. An additional 1 ml of each extract was added to the appropriate scent-detection vial before a new dog/handler team was evaluated. Three dogs (D, E, F) using one handler were evaluated with 20 repetitions each, and the data were taken over a 1 wk period.

**Statistical Analysis**

The percentage of positive or false positive indications was calculated for each scent based on 20 repetitions with each dog except for the hotel room experiment, which had 6 repetitions with each dog. Data were then arcsine square root transformed and analyzed by two-way analysis of variance, with main effects as the dogs and the scents in the scent-detection vials. Means were separated with Student-Newman Keuls (P < 0.05; SAS Institute, 2003).
Results

General Household Pest Experiment

Two-way analysis of variance determined the scent of household pests in scent-detection stations significantly affected the dogs’ responses (df = 4, 3, 8, 380; $F = 3211; P < 0.0001$). There were no significant differences among the four dogs (df = 4, 3, 8, 380; $F = 2.11; P = 0.098$). There was a significant interaction between household pest scents and the tested dogs (df = 4, 3, 8, 380; $F = 2.11; P = 0.0156$), because one dog was less accurate in finding bed bugs when the insects were present. Dogs trained to locate the scent of live bed bugs and viable bed bug eggs were able to distinguish live bed bugs from other household pests, including carpenter ants, cockroaches, and termites (Table 1). When live bed bugs were present in scent-detection stations, the dogs averaged ~98% accuracy in locating them. There were no false positives for any of the dogs; dogs did not indicate at any scent-detection station that did not contain bed bugs. With dogs A, B, and D there were no false positives, no missed indications, and the dogs found the bed bugs every time the insects were present. The positive indications for dogs A, B, and D were significantly higher than the positive indications for dog C as well as the false positives for all dogs (df = 7, 392; $F = 1897.47; P < 0.0001$). There were no false positives for dog C either, but it failed to detect the bed bugs twice during twenty repetitions.

Bed Bug Debris Experiment

Two-way analysis of variance determined the scent of bed bug materials in scent-detection stations significantly affected the dogs’ responses (df = 5, 2, 10, 342; $F = 677; P < 0.0001$). There were no significant differences among the three dogs (df = 5, 2, 10, 342; $F = 0.53; P = 0.59$), and there was not a significant interaction between bed bug debris scents and the tested dogs (df = 5, 2, 10, 342; $F = 0.53; P = 0.87$). Dogs trained to locate the scent of live bed bugs and viable bed bug eggs were able to distinguish the live bed bugs and viable eggs from other
bed bug debris, including bed bug feces, dead bed bugs, and cast skins (Table 2). Dogs were significantly more accurate in locating live bed bugs than they were in locating viable bed bug eggs (df = 5, 174; F = 267; P < 0.0001), but their mean positive indication rate on viable bed bug eggs was still high at 90%. The dogs had an average false positive rate of 3% on bed bug feces, with no false positives on any other scent. All three dogs located the live bed bugs every time the insects were present, giving them a perfect positive indication rate on live bed bugs. Each of the three dogs missed the viable bed bug eggs two times out of twenty repetitions. The overall positive indication rate was the same for each dog at 95%, which was significantly higher than the false positive rates (df = 5, 354; F = 657; P < 0.0001). When live bed bugs and viable bed bug eggs were not present, there was no significant false positive rate although dog A did have two false positives on bed bug feces.

Hotel Room Experiment

Two-way analysis of variance determined the source of the scent (whether the vials contained male or female bed bugs at densities of one, five, or ten) did not significantly affect the dogs’ responses (Table 3) (df = 5, 2, 10, 36; F = 1.0; P = 0.4317). There were no significant differences between the three dogs (df = 5, 2, 10, 36; F = 1.0; P = 0.3779). The interaction between the dogs and the scent vials was also not significant ((df = 5, 2, 10, 36; F = 1.0; P = 0.4618). Dogs trained to locate the scent of live bed bugs and viable bed bug eggs were able to shift that ability from the experimental scent-detection stations to the more realistic hotel room situation, with a 98% average accuracy. Dogs A and B were 100% accurate in locating live bed bugs while dog G was 94.4% accurate. Dog G had one missed indication on one of six possible scent vials out of six repetitions; it did not indicate once on the vial containing five female bed bugs. There were no false positives for any of the dogs; dogs did not indicate anywhere that bed bugs were not present.
Pseudoscent Extracts Experiment

Two-way analysis of variance determined the extract in the scent-detection station significantly affected the dogs’ responses (df = 4, 2, 8, 285; $F = 3571; P < 0.0001$), but again there were no significant differences among the three dogs (df = 4, 2, 8, 285; $F = 1.0; P = 0.369$). There was not a significant interaction between the tested dogs and the extracts (df = 4, 2, 8, 285; $F = 1; P = 0.436$). Dogs trained to locate the scent of live bed bugs and viable bed bug eggs always indicated on the pentane extract (Table 4), but averaged only ~2% on the methanol and had no indications on the acetone, water, or blank scent-detection stations. All dogs averaged 100% indication on the pentane extract which was significantly higher than all other extracts. Dog B had a 5% indication rate on methanol extract. The pentane pseudoscent we used was stored in a refrigerator at a temperature of 3.3 °C. Three months later the dogs still indicated on it, so as long as it is stored properly the pseudoscent has at least a three month shelf-life.

Discussion

Detector dogs trained to locate live bed bugs and viable bed bug eggs have been used as a tool for pest control operatives. However, in order for them to be effective, the dog must be able to locate the target odor accurately. Dogs trained to locate live bed bugs and viable bed bug eggs had an overall accuracy of 97%, which is similar to previous studies on insect detector dogs. A German wirehaired pointer trained to detect screwworms had an accuracy of 99.7% (Welch 1990). Wallner and Ellis (1976) were able to train three German shepherds to detect gypsy moth egg masses at an accuracy of 95%. Six dogs that were trained to locate live termites had an overall accuracy of 96% (Brooks et al. 2003). Similarly, our dogs were able to discriminate bed bugs from other general household pests that may be found in the same locations, such as German cockroaches, Florida carpenter ants, and eastern subterranean termites. The dogs were also able to differentiate materials of an active infestation (live bed bugs and viable bed bug
eggs) from materials of a possibly inactive infestation (dead bed bugs, cast skins, bed bug feces). In a more realistic situation, dogs were also able to locate live bed bugs hidden throughout hotel rooms. The minimum acceptable standard proposed by Brooks et al. (2003) of a positive indication rate of \( \geq 90\% \) and a false positive rate of \( \leq 10\% \) was achieved by the bed bug-detecting canines we tested.

Although a high positive indication rate is a realistic expectation for detection dogs, a few studies showed that some dogs had a positive indication rate less than the proposed minimum acceptable standard (Brooks et al. 2003). Three dogs that were trained to identify off-flavor pond water compounds (2-methylisoborneol and geosmin) had an overall accuracy of 77\% (Shelby et al. 2004). Dogs trained to locate brown tree snakes hidden in cargo on Guam had an overall accuracy of 70\% (Engeman et al. 1998). These lower positive indication rates could be the result of a variety of different factors such as dog training method, training apparatus used, training maintenance, and length of search time. Environmental factors such as temperature, air flow, handler misinterpretation, and scent accessibility could also have affected the accuracy of the dogs (Moulton 1972, Wallner and Ellis 1976, Ashton and Eayrs 1970, Welch 1990). In our study, the dogs had a high positive indication rate because we controlled as many of these influences as possible. The training method we used was modified from Brooks et al. (2003). Training was maintained twice daily and the length of search time was limited to 40 min or less. Air flow was minimal and temperature was constant due to the indoor test environment, and one handler was utilized in all experiments. If the training methods proposed by Brooks et al. (2003) are used, if training is maintained regularly, and if environmental and human factors are controlled, it is possible for dogs to have a positive indication rate equal to or higher than the proposed minimum acceptable standard.
Sometimes dogs do not indicate when the target odor is present; they show no indication. In our study, all dogs had a 10% no-indication rate on viable bed bug eggs. Dogs trained to respond to a target odor will react only if the target odor meets or surpasses a threshold concentration (Moulton 1972, Settles 2005). The relatively high no-indication rate of our dogs on viable bed bug eggs may be due to low concentration of target odor, although the 90% positive indication rate on the viable bed bug eggs was within the acceptable minimum standard. On the other hand, a dog’s response must also be interpreted by the handler. No indications can be caused by the handler misreading dog behavior, emphasizing the importance of an experienced handler.

A high false positive rate may also be caused by faulty training or misinterpretation by the handler. Brooks et al. (2003) reported on a dog with a 75% false positive rate on termite-damaged wood, when the target insects, termites, were not present. That particular dog was trained on termites and termite damaged wood, when the only target odor was termites. However, dogs trained only on termites had a considerably lower false positive rate. In our study, we believe the false positives recorded for dog A on bed bug feces may have occurred because of bed bug defecation in the scent-detection vials. The feces were removed every 2 or 3 wk from the scent-detection vials, which were used daily for training for dog A. Therefore, dog A was being trained on both target and nontarget odors. Feces were monitored and removed daily before training the rest of the dogs.

Training a dog only on target odors can be difficult, especially if handling of target insects is difficult, as with live bed bugs. The creation of a pseudoscent can make the training of bed bug-detecting dogs easier. A pseudoscent can eliminate the need for dog trainers to handle bed bugs while ensuring the dogs are only being trained on the target odor. The dogs did not
indicate on the acetone or water extract. One dog indicated once on the methanol extract. Pentane seems like the most possible candidate for creating a pseudoscent since all dogs indicated 100% on the pentane extract. It seems that pentane has the ability to contain the target odor of the bed bugs because the dogs indicate on the pentane extract like they indicate on live bed bugs and viable bed bug eggs.

The pentane pseudoscent can be utilized in many different ways. It can be used to train dogs, replacing the live bed bugs that many people are uncomfortable handling. Also, quality control programs are necessary and usually required in order to evaluate whether trained dogs continue to work properly (Doggett 2007). The existence of a pseudoscent would be ideal in this situation. The pseudoscent would allow a technique for quality assurance that could be used in any building, without the possibility of accidentally creating infestations.

Bed bug-detecting canines can be a valuable tool to the industry. They can aid in the detection of early and established infestations. From an economic point of view, locating these infestations can reduce the number of possible lawsuits from customers (Doggett 2007). Instead of hotel managers learning of an infestation due to a customer being bitten, they can seek out the infestations and treat them before customers are affected. Also, because bed bug-detecting canines can be trained only to locate live bed bugs and viable bed bug eggs, the dogs can recheck previously treated rooms to confirm whether or not the treatment was successful.

Our study has shown that dogs can be trained to accurately locate live common bed bugs (Cimex lectularius) and viable bed bug eggs at a positive indication rate \( \geq 90\% \) and a false positive rate \( \leq 10\% \), as proposed by Brooks et al. (2003). Dogs can differentiate the live bed bugs from other general household pests, such as German cockroaches, eastern subterranean termites, and Florida carpenter ants. The dogs can also discriminate live bed bugs and viable bed
bug eggs from other bed bug materials, such as cast skins, feces, and dead bed bugs. The hotel room experiment showed that dogs can locate as few as one bed bug in a hotel room. The production of a pseudoscent would make it easier to train dogs only on the target odor, possibly increasing the accuracy of the dogs. Dogs can be trained to locate cryptic insects that are difficult to uncover visually as long as dogs are trained in a similar manner to the method we used, training is maintained regularly, an experienced handler is used, and nontarget odors are separated from target odors. The ability of carefully trained dogs to accurately locate cryptic insects holds many possibilities; dogs could be used to locate and monitor populations of many important insects, such as Africanized honeybees or the emerald ash borer.
Table 4-1. Percent indication (mean % ± SE) by dogs at scent-detection stations containing live general household pests and live common bed bugs (Cimex lectularius).

<table>
<thead>
<tr>
<th>Dog</th>
<th>Bed Bugs</th>
<th>Ants</th>
<th>Cockroaches</th>
<th>Termites</th>
<th>Blank</th>
<th>Positive(^a)</th>
<th>False Positive(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100 ± 0a</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>100 ± 0x</td>
<td>0 ± 0z</td>
</tr>
<tr>
<td>B</td>
<td>100 ± 0a</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>100 ± 0x</td>
<td>0 ± 0z</td>
</tr>
<tr>
<td>C</td>
<td>90 ± 6.88b</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>90 ± 6.88y</td>
<td>0 ± 0z</td>
</tr>
<tr>
<td>D</td>
<td>100 ± 0a</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>0 ± 0c</td>
<td>100 ± 0x</td>
<td>0 ± 0z</td>
</tr>
<tr>
<td>Mean</td>
<td>97.5 ± 1.76m</td>
<td>0 ± 0n</td>
<td>0 ± 0n</td>
<td>0 ± 0n</td>
<td>0 ± 0n</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in a treatment block followed by the same letter are not significantly different (P=0.05; Student-Newman-Keuls; SAS Institute 2003).

\(^a\) Positive indications are indications by dogs on bed bug scent.
\(^b\) False positive indications are indications by dogs on any scent other than bed bugs.
Table 4-2. Percent indication (mean % ± SE) by dogs at scent-detection stations containing bed bug materials, live common bed bugs and viable bed bug eggs (*Cimex lectularius*).

<table>
<thead>
<tr>
<th>Dog</th>
<th>Live Bed Bugs</th>
<th>Viable Bed Bug Eggs</th>
<th>Feces</th>
<th>Cast Skins</th>
<th>Dead Bed Bugs</th>
<th>Blank</th>
<th>Positive&lt;sup&gt;a&lt;/sup&gt;</th>
<th>False Positive&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100 ± 0</td>
<td>90 ± 6.88</td>
<td>10 ± 6.88</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>95 ± 3.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 ± 1.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>100 ± 0</td>
<td>90 ± 6.88</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>95 ± 3.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>100 ± 0</td>
<td>90 ± 6.88</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>95 ± 3.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>100 ± 0x</td>
<td>90 ± 6.88y</td>
<td>3.33 ± 2.34&lt;sup&gt;z&lt;/sup&gt;</td>
<td>0 ± 0z</td>
<td>0 ± 0z</td>
<td>0 ± 0z</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in a treatment block followed by the same letter are not significantly different (P=0.05; Student-Newman-Keuls; SAS Institute 2003).

<sup>a</sup> Positive indications include indications of dogs on live bed bug and viable bed bug egg scents.

<sup>b</sup> False positive indications include indications of dogs on any scent other than live bed bugs or viable bed bug eggs.
Table 4-3. Ability of dogs to locate varying numbers of live male and female bed bugs (*Cimex lectularius*) in hotel rooms.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Number of Female Bed Bugs</th>
<th>Number of Male Bed Bugs</th>
<th>Positive$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>A</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>B</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>G</td>
<td>100 ± 0</td>
<td>66.7 ± 33.33</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Mean</td>
<td>100 ± 0</td>
<td>88.9 ± 11.11</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

There were no significant differences at all variables (P=0.05; Student-Newman-Keuls; SAS Institute 2003).

a Positive indications include indications of dogs on live bed bug and viable bed bug egg scents

b There were no false positive indications
Table 4-4. Percent indication (mean % ± SE) by dogs at scent-detection stations containing chemical rinses of live common bed bugs (*Cimex lectularius*).

<table>
<thead>
<tr>
<th>Dog</th>
<th>Pentane</th>
<th>Methanol</th>
<th>Acetone</th>
<th>Water</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>E</td>
<td>100 ± 0</td>
<td>5 ± 5</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>F</td>
<td>100 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Mean</td>
<td>100 ± 0a</td>
<td>1.67 ± 0b</td>
<td>0 ± 0b</td>
<td>0 ± 0b</td>
<td>0 ± 0b</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different (P=0.05; Student-Newman-Keuls; SAS Institute 2003).
Figure 4-1. Layout of furniture in hotel rooms, locations where bed bugs were hidden, and path used for searching the rooms.
The common bed bug, Cimex lectularius (L.), occurs in aggregations (Usinger 1966), consisting of bed bugs of all life stages, feeding status, and mating conditions (Johnson 1942, Reinhardt and Siva-Jothy 2007). Bed bugs are found in field populations at a 1:1 sex-ratio (Johnson 1942, Newberry and Jansen 1986, Stutt and Siva-Jothy 2001), suggesting that females cannot avoid males and traumatic insemination (Reinhardt and Siva-Jothy 2007). Traumatic insemination is a method of copulation where the male uses his genitalia to pierce the abdomen of the female and injects sperm outside of the reproductive tract, causing a physical wound (Usinger 1966). Multiple traumatic inseminations benefit male bed bugs because they results in last-male sperm precedence but cause females to have a shorter life-span and reduced fecundity (Stutt and Siva-Jothy 2001). Therefore, if females stay in aggregations with high proportions of males, they are exposed to multiple traumatic inseminations and the undesirable consequences of reduced longevity and fewer fertile eggs.

Although bed bugs usually occur in aggregations, there are times when bed bugs may be found alone, dispersed from the aggregations. I found that females are the dispersal stage of the common bed bug. In my experiments, recently fed females left aggregations and were found alone at a much higher rate than any other life-stage, especially when the proportion of males was high. Mating occurs in the first 36 hours after feeding, and fully engorged females cannot avoid traumatic insemination (Stutt and Siva-Jothy 2001). It is likely that the dispersing females had already mated at least once. As a result, when females leave aggregations, they are likely laying eggs away from other aggregations. When the eggs hatch, nymphs emerge, produce aggregation pheromone (Siljander et al. 2007), and start new aggregations.
Female bed bugs not only avoided males by dispersal, but also avoided males by aggregating with each other in female-biased aggregations. A similar situation is found with the African damselfly, Platycypha caligata, where females form groups to avoid harassment from courting males (Martens and Rehfeldt 1989). Unlike nymphs and males, female bed bugs do not produce any aggregation pheromone (Siljander et al. 2007). This lack of aggregation pheromone production can be beneficial because males may not be able to locate the females leaving male-biased aggregations to avoid multiple traumatic inseminations. Female-biased aggregations may result from the lack of a force driving females to disperse from these aggregations. This allows females to benefit from female-biased aggregations, for instance by reduced water loss (Benoit et al. 2007) and greater protection from multiple traumatic inseminations.

As females leave aggregations and either remain alone or form their own female-biased ones, the aggregations they left behind become increasingly male-biased. In my experiments, as the proportion of males in aggregations increased, the proportion of lone males also increased. This is possibly driven by males searching for females to mate with. Because males release aggregation pheromone (Siljander et al. 2007), females may potentially locate males when copulation is necessary, but can continue to avoid males once females leave the aggregations.

Because females are dispersing from aggregations, and because it is likely that these females have been inseminated, a method to detect bed bugs becomes even more crucial. Canines trained to detect live bed bugs could differentiate bed bugs from other general household pests at 97.5% with no false positives on any of the other insects used. It is important that they do not indicate on other household pests that could occur in the same area because the treatment for bed bugs is much different than the treatment for other insects that could be there as well. In the bed bug materials experiment, dogs had a 100% positive indication rate on live bed bugs and
a 90% positive indication rate on viable bed bug eggs. It is important that the dogs only indicate on live bed bugs and viable bed bug eggs because those are the only true signs of an active infestation. Indicating on dead bed bugs, bed bug feces, or cast skins is not efficient because they could be remnants of a past infestation that was eliminated.

When vials containing live bed bugs were hidden in hotel rooms, dogs averaged a 98% positive indication rate. This experiment shows that dogs can locate bed bugs in a more realistic situation, when variables such as temperature and air flow cannot be controlled. Of all compounds tested as a pseudoscent, pentane is the best possible candidate. Pentane was the only nonpolar compound tested, which seems to be important in holding the scent of the bed bugs. Properly trained canines can efficiently locate the common bed bug, and the manufacture of a pseudoscent would greatly help with training the dogs.
LIST OF REFERENCES


Cooper, R. 2007. Are bed bug dogs up to snuff? Pest Control 75(8); 49-51.


BIOGRAPHICAL SKETCH

I was born and raised in Cincinnati, Ohio. I attended McAuley High School, where I graduated in 2002. I proceeded to attended The College of Mount St. Joseph until 2006, when I received my Bachelor of Science degree in biology. I played competitive soccer throughout high school and college. I moved to Gainesville, Florida, in August 2006 to pursue my Master of Science degree in entomology.