

**Evaluation of Multiple Insecticidal Products for Control of  
the Common Bed Bug (*Cimex lectularius* (L.))**

**David Joseph Moore II**

**Thesis submitted to the faculty of the Virginia Polytechnic Institute and State  
University in partial fulfillment of the requirements for the degree of**

**Masters of Science in the Life Sciences  
in  
Entomology**

**Approved by:**

**Dini Miller**

Jeffrey Bloomquist

Sally Paulson

**November 16, 2006**

Blacksburg, VA

Keywords: Bed bugs, pyrethroids, repellency, hydroprene, field evaluation

Copyright 2006, David J. Moore II

# **Evaluation of Multiple Insecticidal Products for Control of the Common Bed Bug (*Cimex lectularius* (L.))**

**David Joseph Moore II**

## **Abstract**

The common bed bug has reemerged as a major pest in the United States. Pest management professionals need reliable up-to-date information on how to manage bed bug infestations. My study was intended to evaluate the efficacy of several insecticides currently labeled for bed bug control.

In product efficacy tests, field strain bed bugs were found to be 99-450 times less susceptible than laboratory strain bed bugs to several pyrethroid products. The non-pyrethroid products tested, chlorfenapyr and a non-toxic desiccant dust, killed laboratory strain bed bugs, but were extremely slow acting taking greater than 9 days to kill 50%. None of the insecticides tested, including the pyrethroids, were repellent to laboratory or field strain bed bugs.

A field test was conducted comparing 2 pesticide treatments regimens (traditional and novel) for bed bug control in low income apartments. Both the traditional and novel combinations caused significant reductions in bed bug populations. Both treatments reduced the number of bed bugs by the end of the test period, but neither treatment combination completely eliminated the bed bug infestations, even after an average of 1.3 gallons of product was applied in each apartment.

Laboratory assays were conducted to determine the effect of hydroprene exposure on bed bug development. Although hydroprene did not appear to interfere with nymphal development, fifty percent of the bed bugs died during the final molt. The bed bugs which survived to adulthood showed no reduction in fecundity when compared to control groups.

## **Acknowledgment**

This Thesis is by far the most significant accomplishment of my life. It would have been impossible without the people who supported and believed in me. Most of all, I would like to extend my most heartfelt gratitude to my advisor and mentor Dr. Dini Miller, for her patient guidance and enthusiasm throughout my program. I would also like to thank the members of my committee, Dr. Jeffrey Bloomquist and Dr. Sally Paulson for their input and encouragement.

I am grateful to the members of the Dodson Urban Pest Management Laboratory, specifically Tim McCoy and Tamara Sutphin. I would not have finished without their help. I would like to sincerely thank Tim McCoy, who was like a second mentor to me. I am indebted to you and Dr. Miller for helping me grow as a person and as a scientist.

Everything I know about pest control could be traced back to the wonderful pest management companies I worked for in the past. Bob Conner of Bob's Termite and Pest services taught me the basics I needed to succeed. Then Barry and Cindy Robinson of ServisPros allowed me to expand my knowledge and encouraged me to pursue my education. Their encouragement is one of the reasons why I pursued this degree.

My love goes out to all my friends and family. Dad, thank you for always pushing me. More importantly, thank you for letting me fall and helping stand me back up when needed. Lady, Thank you for keeping me grounded. You have always been honest and you kept me calm through this process. Thank you for all your support Danielle. You were always there with uplifting spirits when I needed someone to talk to. I would also like to thank everyone else I consider to be close friends and family. You were all there when I needed encouragement or to be put back into place. Thank you all for pushing me forward and not allowing me to step back.

## **Table of Contents**

<b>List of Figures.....</b>	<b>v</b>
<b>List of Tables.....</b>	<b>vi</b>
<b>Chapter One</b>	
<b>Introduction.....</b>	<b>1</b>
<b>Chapter Two</b>	
<b>Literature Review .....</b>	<b>5</b>
<b>Chapter Three</b>	
<b>Efficacy and Repellency of Insecticides Labeled for Bed Bug Control.....</b>	<b>35</b>
<b>Chapter Four</b>	
<b>The Evaluation of Two Treatment Combinations for the Control of Bed Bugs in Low Income Apartments .....</b>	<b>60</b>
<b>Chapter Five</b>	
<b>Evaluation of Hydroprene on Bed Bug Mortality and Fecundity .....</b>	<b>77</b>
<b>Chapter Six</b>	
<b>Summary.....</b>	<b>100</b>
<b>List of References.....</b>	<b>104</b>

## List of Figures

<b>Figure 2.1.</b> Dorsal view of nymphal bed bug anatomy.....	30
<b>Figure 2.2.</b> Dorsal View of an adult bed bug anatomy.....	31
<b>Figure 2.3.</b> Ventral View of an adult bed bug anatomy.....	32
<b>Figure 2.4.</b> Ventral view of adult bed bug thorax.....	33
<b>Figure 2.5.</b> Genitalia of a male and female bed bug.....	34
<b>Figure 3.1.</b> Diagram of repellency bioassay arena- Non-choice test.....	50
<b>Figure 3.2.</b> Diagram of repellency bioassay arena- Choice test.....	50
<b>Figure 3.3a-3.3e.</b> Mean percent ( $\pm$ SE) of laboratory strain bed bugs aggregating on treated panels in non-choice repellency assays.....	51
<b>Figure 3.4a-3.4e.</b> Mean percent ( $\pm$ SE) bed bug response to treated and untreated panels in choice repellency assays.....	56
<b>Figure 3.5a-3.5e.</b> Mean percent ( $\pm$ SE) bed bug response to treated and untreated panels in pyrethroid bioassays.....	61
<b>Figure 4.1:</b> This is a chart of the mean number of bed bugs observed on each different test date for each of the three treatment regimens.....	76
<b>Figure 5.1.</b> Diagram of hydroprene application to filter paper.....	92
<b>Figure 5.2.</b> Filter paper placement inside vial for bed bug exposure top view.....	92
<b>Figure 5.3.</b> Placement of bed bugs in vials, Hobo (records temp. and R. H.), and salt water solution (humidity regulation) inside storage container.....	93
<b>Figure 5.4.</b> Image of a hydroprene exposed bed bug which died mid-molt between 5 <sup>th</sup> instar and adult.....	94

<b>Figure 5.5.</b> Image of a hydroprene treated bed bug which died after completing its final molt. However, the bed bug did not completely sclerotized. The pale abdominal region indicates incomplete sclertization.....	94
<b>Figure 5.6.</b> Scanning electron microscope (SEM) image of a normal male bed bug paramere.....	95
<b>Figure 5.7.</b> Scanning electron microscope (SEM) image of a normal female bed bug's genitalia which molted into an adult, but did not completely sclerotize with the paramere deformed.....	96
<b>Figure 5.9.</b> Scanning electron microscope (SEM) image of a hydroprene exposed male bed bug which molted into an adult, but did not completely sclerotize. The paramere was 25% shorter than male controls.....	97
<b>Figure 8.</b> Scanning electron microscope (SEM) image of a hydroprene exposed male bed bug which molted into an adult, but did not completely sclerotize with the paramere deformed.....	98
<b>Figure 5.10.</b> Scanning electron microscope (SEM) image of a hydroprene exposed female bed bug which molted into an adult, but did not completely sclerotize. The female genitalia was completely deformed.....	99

## **List of Tables**

<b>Table 3.1.</b> Time to mortality of susceptible strain and field adult bed bug confined on insecticide treated hardboard panels.....	48
<b>Table 4.1.</b> Number of live bed bugs observed in each apartment unit by location.....	74
<b>Table 4.2.</b> Products applied in each treatment combination per unit per application.....	75
<b>Table 5.1.</b> Comparison of mean number of eggs produced and hatched in each treatment group.....	90

## **Chapter One**

### **Introduction**

The common bed bug, *Cimex lectularius* (Linnaeus), is a blood-feeding parasite of humans, bats, and several domesticated animals (Usinger 1966). Bed bugs are typically active at night and hide during the day in cracks, crevices, and other harborage locations (Usinger 1966). At the turn of the 20th century, the bed bug was a common household pest in the U.S. and throughout the world (Ebeling 1975). Bed bugs were frequently encountered in quality hotels and motels as well as less respectable establishments and were easily transported into a traveler's home via their clothes, vehicles, or luggage. Once inside the home, bed bugs could find harborage in furniture, cracks and crevices in the floor or walls, behind wallpaper, or under carpeting (Krueger 2000).

The importance of the bed bug as a household pest began to diminish in the 1940s and '50s with the use of pyrethrum insecticides and DDT (Ebeling 1975). In the latter half of the 20th century, the bed bug was almost eradicated in the United States with only a few persistent populations surviving in locations where living conditions were primitive or unsanitary (Ebeling 1975).

However, in the early 1990s pest management professionals began to see an increase in bed bug infestations across the nation (Krueger 2000). While the exact reason for this increase in bed bug activity is not known, there are several factors that may have contributed to the resurgence of bed bugs in the U.S. It has been suggested that international travel from developing nations has increased the distribution of bed bugs throughout the world (Potter 1997). However, international travel has been taking place for several decades and the bed bugs have only recently (1990s) become recognized as a widespread problem. Another possibility for the



bed bug increase in activity has been the reduction in residual insecticide use indoors. Due to concerns about human exposure risk, routine interior applications of spray insecticide have been greatly reduced in favor of integrated pest management (IPM), where insecticides are applied only on an as-needed basis. So, in the absence of residual sprays bed bug populations have established new infestations unchecked. IPM also uses insecticide baits as the primary method for controlling crawling insects. However, insecticide baits do not affect blood sucking parasites. One final possibility is that bed bug populations across the world have become resistant to insecticide products used in developing nations and the U.S. If this is the case it is reasonable to suggest that the increasing population pressure has led to the widespread dissemination of bed bugs throughout the world.

With bed bug infestations making a comeback around the globe, the potential health issues associated with bed bugs becomes a concern (Boase 2004). Although bed bugs have never been implicated as a vector of human disease, bed bugs are suspected carriers of leprosy, oriental sore, Q-fever, and brucellosis (Dolling 1991). There is also evidence that the Hepatitis B virus may be mechanically transmitted to humans in bed bug feces or when bed bugs are crushed during feeding (Blow et al. 2001). Allergic reactions have also been documented in response to bed bug bites (Potter 1997) and it is suspected that bed bugs may also be a contributing factor to asthma in people living with large populations of bed bugs (Fu and Shong 1995).

Compounding the health issues associated with bed bugs is the fact that they are difficult to manage. It has been documented that bed bugs developed resistance to a number of the insecticides previously used for their control. These chemicals include DDT, methoxychlor, dieldrin, aldrin and other organochlorine compounds (Busvine 1980; Gaaboub 1971), carbaryl, endrin, gamma BHC (Radwan et al. 1972), malathion and other organophosphates, and sodium

fluoride (Feroz 1974). Many of these older chemistries have been banned by the U. S. Environmental Protection Agency and are no longer available for bed bug treatment.

Because bed bugs have not been a problem in the U.S. for over 40 years, few of the newer insecticide products are labeled for bed bug control. The majority of insecticide products that are labeled for the treatment of bedbugs are either natural pyrethrins or pyrethroids. Because these active ingredients are from the same chemical class, resistance to the formulated products could easily develop. In addition, most pyrethroid-type chemistry has been shown to be repellant to crawling pests such as cockroaches, (Ebeling et al. 1966) termites (Smith 1979), and ants (Knight and Rust 1990). The potential for repellency suggests that aggregations of bed bugs may scatter if they are treated with these products directly or that individual bed bugs may avoid surfaces treated with pyrethroids. These behavioral responses would hinder pyrethroid efficacy as a control agent for bed bugs. Pyrethroid products are commonly used to treat for bed bugs today, even though the bed bugs' response to these products has not been empirically determined.

There are also several non-pyrethroid products that are labeled for bed bug control. These include Gentrol (hydroprene), an insect growth regulator, Steri-Fab a non-residual mattress spray that kills bed bugs on contact, and Natural Insect Control dust (N.I.C. 325, limestone, and corn bran) which is a desiccant dust labeled for mattress and crack and crevice treatment. Another product in common use is Phantom, a residual crack and crevice spray that is not specifically labeled for bed bugs but has a site label that allows for the indoor treatment for crawling insects. The active ingredient in Phantom is chlorfenapyr (0.5%), a pyrrole insecticide that functions as a stomach toxicant where the insecticide metabolites produce the toxic effects. These products are also being used by the pest management industry, but like the pyrethroids, there is no published efficacy data to indicate that these products actually kill bed bugs.

Due to concerns about possible scattering of bed bug populations many researchers have been recommending the use of non-repellant products in combination with sanitation and visual monitoring to create an integrated treatment program for bed bug management. However, these recommendations have been based solely on experience with other crawling insects such as cockroaches (Ebeling et al. 1966) and ants (Knight and Rust 1990). Pyrethroid products have not been evaluated for repellency to bed bugs and no other products have been evaluated for efficacy in controlling bed bugs either in the field or the laboratory. There is a great need to determine if any of these labeled products actually kill bed bugs.

## **Chapter Two**

### **Literature Review**

#### **Bed Bug Distribution and History**

**Bed Bug Distribution.** The common bed bug, *Cimex lectularius*, is believed to have originated from an ancestral biotype in the Middle East during the period when humans and bats cohabitated in caves (Sailer 1952). Although the exact period is unknown, Cimicids that fed preferentially on humans could have evolved as early as 100,000 years ago when Neanderthal man was living in caves or as late as 25,000 years ago prior to the domestication of fire (Oakley 1956). When the more advanced nomadic human populations began to expand into new locations the bed bugs hitched a ride and became established in human dwellings (Mellanby 1935). When human populations gradually settled into villages, and later cities, bed bugs also moved with them into these new habitats (Usinger 1966). There has been a trend for increasingly larger cities since 4000 BC (Adams 1960), which allowed for bed bugs to expand their range over time (Kemper 1936). Bed bugs originated in the Middle East, moved to Africa, Asia, and then the Mediterranean, and then were introduced to the rest of Europe as people began to spread out across the continent (Horvath 1914, Usinger 1966). Bed bugs spread throughout Europe and were reported in Germany by the eleventh century, France during the 1200s and England by 1583 (Kemper 1936). Bed bugs were brought over with the European colonists as they migrated to the new world (Jones 2004). Usinger (1966) wrote that Mouffet (1634) described the earliest report of bed bugs in England was in 1583 when Thomas Penny was called to treat bed bugs for two Noble ladies of Mortlake. During the early 20<sup>th</sup> century, as central heating became more prevalent (Johnson 1942) bed bug infestations continued to increase both in Northern Europe and North America. Today bed bugs have a world-wide distribution and are found living among all developed human populations (Hwang et al. 2005).

**Bed Bug History.** References to bed bugs appear in some of the earliest recorded history. Aristotle referenced bed bugs in *Historia Animalia* (384-322 B.C.). Usinger (1966) describes how Dioscorides of Cilicia used a liquid homogenate of bed bugs as an anti-venom during the time of Nero (54-68 A.D). However, the bed bug extract he created often produced worse consequences (intense illness or death) than the diseases he was trying to cure. Bed bugs were mentioned in the Talmud (written between 220 C.E. and 600 C.E.; Bodenheimer 1929) and in the “Acts of John” (Leucius, 2<sup>nd</sup> century A.D; Schneemelcher 1989). The presence of bed bugs was documented in Greece in 499 B.C., Italy in 77 A.D., and in China by 600 A.D (Usinger 1966). Due to the prevalence of bed bugs in human history, the statement “the control of bed bugs has tested man’s ingenuity for centuries” (Usinger 1966), is still true today.

Because bed bugs have co-existed with humans for centuries, many common names, phrases, beliefs, and folk remedies have been created surrounding them. Bed bugs are known to have over fifty common names (Usinger 1966). The English language names include wall-louse, red coats, pursuer, wall flounder, and the mahogany-flats (Kemper 1959). In addition to the various common names, there are a number of folk sayings that are related to bed bugs, these include "crazy as a bed bug "or "snug as a bug in a rug," which are used to describe people or conditions. It is often difficult to explain the exact meaning of some of these sayings (Taylor 1956). Even the statement “sleep tight don’t let the bed bugs bite” does not have a clear origin. It has been suggested that the “sleep tight” refers to tightening the ropes on 18<sup>th</sup> century mattresses to prevent the mattresses from sagging and thus keeping bed bugs from contacting the sleeping traveler. However, this interpretation is controversial and may simply be a permutation of the Susan Bradford Epps (1866) diary reference “sleep tight and wake bright” (Epps 1968). Other folk beliefs associated with bed bugs include the idea that “bed bugs are a warning of a

fight” (New York Folklore Quart., 4:163 in Usinger 1966) or that “if a bed bug has seen daylight between August 15 and September 8 that the bed bug will be strong enough to penetrate seven walls” (J. Amer. Folklore 61: 278 in Usinger 1966). Some of the more realistic beliefs have to do with how bed bugs spread from home to home. One common belief was that the return of migrating swallows brought bed bugs into a home (Hand 1956). This belief has a basis in fact because swallows are the host of swallow bugs, another Cimicid that will feed on humans if the swallow host departs. Usinger (1966) wrote that Rupp (1946) found bird bugs associated with Chimney swifts have also been mistaken for bed bugs, as have bat bugs (Usinger 1966). Both bird bugs and bat bugs are capable of infesting human dwelling and will do so readily if their preferred host population departs or is eliminated (Ebeling 1975).

Bed bugs have long been the target of a number of folk remedies. Some may have been reasonably effective while others, though creative, were probably useless. One ancient remedy was to hang the feet of a dead stag or rabbit at the foot of the bed (Cowan 1865). The hanging foot remedy most likely did not work. In the *Geoponika* (Owen 1806), according to Varro and Columella, extracts of bitter lupin or wild cucumber were part of a procedure for killing bed bugs during the classical Mediterranean period. The plant bugbane (*Actaea cimicifuga*) has also been named as a treatment for bed bugs as a repellent (Foster 1999). These plant extracts may have been somewhat effective for killing bed bugs. Another plant that demonstrates true potential for bed bug control is the snap bean (*Phaseolus vulgaris*). In the Balkans, and former Southern Rhodesia, the leaves of these bean plants were left overnight on the floor under the beds of infested rooms. The bed bugs become entangled in the hairs on the bean leaves during the night. In the morning the leaves were swept up and burned to destroy the bed bugs (Richardson 1943). Bed bug traps were widely used in the UK and France during the 19th century. These traps

consisted of flat, woven basket-work panels that were placed behind the bolster of the bed, and removed each morning. The bed bugs were then shaken out and killed (Okey, 1930). A folk method of bed bug prevention was recorded by Merton (1955) who wrote of a traveler who controlled bed bugs by placing pans of water under the legs of the bed. The treatment failed because the bed bugs climbed up the ceiling over the individuals and dropped down onto the bed. The traveler was then forced to open an umbrella in order to prevent the bed bugs from feeding on him. However, the umbrella also failed to prevent bed bug bites.

In spite of all of the recorded history of cohabitation with humans, bed bugs are still “the bug that nobody knows” (Usinger 1966). The reason for this bed bug denial on the part of humans is that there is a social stigma associated with bed bug infestations (Krinsky 2002). Many people believe that bed bugs only infest lower income housing. Bed bug infestations are also believed to occur only in areas of overcrowding and unsanitary living conditions (Gbakima et al. 2002, Newberry and Jansen 1986). Therefore, you can only get bed bugs if you spend time in these undesirable locations (Ryan et al 2002). However, this has never been true. Bed bugs do not discriminate and will infest every social class and nation across the world. The bed bug’s willingness to infest any human environment has been confirmed by the bed bug resurgence that has taken place in industrialized nations over the past eight years (Doggett et al. 2004, Hwang et al 2005, Gangloff et al. 2006, Harlan 2006a). During the 1990’s bed bugs in the United States, Australia, Singapore, and Europe were reported infesting five star hotels and other expensive tourist locations with no signs of the number of infestations slowing down (Boase 2004).

## **Bed Bug Resurgence**

Due to an increased use of DDT and malathion, bed bugs were virtually eliminated in the United States after World War II. However, since the late 1990s, bed bugs have made a resurgence throughout the country (Cooper and Harlan 2004, Potter 2004, Gooch 2005, Harlan 2006a, Cooper 2006). While the exact reason for this resurgence is unknown, several factors may have contributed to the increase of bed bug infestations across the United States.

A general lack of awareness by the pest control industry, health care professionals, and the public at large helped the spread of bed bugs (Cooper 2006). Prior to the late 1990s, bed bugs had become so scarce that Snetsinger (1997) reported it was hard to find specimens to use in an entomology class. Because bed bugs were so rare, many pest control operators were unaware of how to properly identify and treat for bed bugs (Cooper 2006, Potter 2006). The general public's lack of awareness on how to properly identify bed bugs contributes to the spread of bed bug infestations (Cooper 2006). The medical community is also unaware of how to properly identify bed bug victims (Cooper 2006). When an individual wakes up with bites, bed bugs should be one of the first considerations. However the bed bug bites are often misdiagnosed, allowing a bed bug population to persist (Cooper 2006).

Another possible reason for the increase in activity by bed bug is the reduction of residual insecticides used indoors. Due to concerns about human exposure risk, integrated pest management (IPM) has largely replaced routine interior applications of residual spray insecticides (Cooper and Harlan 2004). Integrated pest management uses insecticide baits as the primary method for controlling crawling insects (Cooper and Harlan 2004) and these insecticide baits do not affect blood sucking parasites. Therefore, bed bug populations have established new infestations unchecked by the presence of residual sprays.



Bed bugs are no longer associated with places with poor living conditions. They have spread through almost every country, most cities, and across social and economic strata (Cooper and Harlan 2004). A contributing factor to their spread is the increase in international and domestic travel (Potter 1997, Kells 2006a). This increase has sped the distribution of bed bugs throughout the world. Hotels with a high rate of occupancy and quick turnover allow bed bugs to spread quickly through an area (Kells 2006a). International travel cannot, however, be the sole cause of the increase in bed bug distribution, as the increase in distribution has only been recognized since the late 1990s and international travel has been conducted for several decades without incidence.

Seasonal migrations of workers may contribute to the spread of bed bugs (Kells 2006a). When these migrant workers move from place to place, they transport bed bugs with their belongings helping to spread the bed bugs over vast distances. When these bed bugs are transported to the migratory workers' community, the residents stop trying to eliminate the bed bugs from their home. These residents instead switch to bed bug suppression (Kells 2006a). These populations become reservoirs for bed bug growth and could help add pesticide resistance to bed bug populations (Kells 2006a).

Bed bug populations across the world may have become resistant to insecticide products used in developing nations and the United States. If this is the case it is reasonable to suggest that increasing population pressure has led to the widespread dissemination of bed bugs throughout the world. Bed bug resistance to multiple insecticides has been documented many times, which can be a contributor for bed bug infestations if resistant reservoir populations are allowed to persist (Busvine 1980; Gaaboub 1971, Radwan et al. 1972, Feroz 1974, Kells 2006a).

The United States has had a steep increase in bed bug infestations over the past few years, with a 10-fold increase in bed bug treatments in recent years. There is little to indicate that the spread of bed bug populations is slowing down (Cooper 2006, Harlan 2006a, Potter 2006). When it comes to bed bugs, especially in regards to the increase of infestations across the United States, there are more questions than answers (Cooper 2006). Due to this lack of knowledge, basic and applied research is needed for bed bug control.

### **Bed Bug Biology and Behavior**

**Morphology.** *Head.* The head of the common bed bug is cylindrical and smaller than the prothorax (Krinsky 2002). The multifacet eyes are knoblike, arising from the lateral margins of the head capsule and protruding from the sides of the head. The ventral wall of the head capsule is membranous except for 2 ventral plates separated along the median line (Usinger 1966). Ocelli are not present (Krinsky 2002).

The antennae arise from the underside of the head, between the eyes and the clypeus (Figure 1; Gunn 1933). Bed bug antennae are always 4 segmented (Harlan 2006a) and half the length of the legs (Gunn 1933). The first antennal segment, the scape, is on a small protuberance between the eyes and the clypeus (Usinger 1966). The second antennal segment, the pedicel, is long and cylindrical with a pale ring below the point of attachment (Usinger 1966). The third and fourth segments, the flagellum of the antenna are both thinner than the second segment, with the fourth segment the thinnest and slightly filiform (Usinger 1966).

The clypeus is partially defined by lateral sutures which do not define the clypeus posteriorly (Figure 2). Paraclypeal lobes are on either side of the clypeus, which surrounds the base of the rostrum and the maxillary lobes.

The labrum appears as free sclerites at the end of the anterior margin of the head. The labrum is semicircular and semi-elliptical with a clear labral suture dividing it from the clypeus (Usinger 1966). Below the labrum on the ventral side of the head is the labium, which starts at the edge of the anterior end of the bed bug head.

The labium arises on the ventral side of the head from within the anterior margin of the head beneath the labrum (Figure 3). The labium is 3 segmented (Harlan 2006a) with a small sclerite at its base. The sclerite is possibly a vestigial fourth segment (Usinger 1966). When at rest, the labium, the outer sheath of the proboscis (Gunn 1933), which contains the fascicle of stylets, sits in a longitudinal groove on the ventral side of the thorax and extends into the prothorax (Usinger 1966). The mandibular and maxillary stylets are dorsally enclosed by the labium (Krinsky 2002). The tip of the labium consists of two lobes bearing sensory papillae (Usinger 1966).

*Thorax.* The prothorax is broad and wing-like on its posterior edges, with a concave anterior margin which surrounds the head (Figure 4; Usinger 1966). Dorsally, the prothorax consists of a single plate. Ventrally, the prothorax is mostly membranous with the subcoxa surrounding the coxa. The two coxa are separated from each other by a distinct suture at the median line. Posterior to each coxa is the mesothoracic spiracle which lies in a small sclerite surrounding it.

The mesothorax lies posterior to the prothorax, with its dorsal side consisting of a single plate (Usinger 1966). The mesothorax is covered dorso-ventrally by hemelytral pads (Krinsky 2002), which bed bug nymphs do not possess. Ventrally, the mesothorax is largely membranous, with the coxa surrounded by mesothoracic subcoxa. These subcoxae are broadly separated mesally and bear the trochantins, which are attached closely to the subcoxal condyle, the point at which

an appendage is articulated into the cavity. Between the subcoxae are two longitudinal plates (sternites). The metathoracic spiracles lie behind the subcoxae in small sclerites (Usinger 1966).

The metathorax consists of a single plate dorsally. The ventral side of the metathorax consists mainly of membranous material and a metathorax subcoxa which surrounds the coxa. However, unlike the metathorax, there are no sternal plates present on the mesothorax (Usinger 1966).

*Legs.* All of the normal leg segments are present on bed bugs. The tarsus is a 2 segmented in nymphs and 3 segmented in adults, with the terminal segments as long as the other 2 segments combined. There are always two simple claws present in both the nymphs and the adults. On the posterior side at the apex of the tibiae there are short soft bristles (Gunn 1933).

*Abdomen.* The abdomen consists of eleven segments and can expand tremendously during blood feeding (Krinsky 2002). The first three segments are only partially sclerotized while the remaining eight segments are sclerotized dorsally (Usinger 1966). Typically, the bed bug abdomen is flattened dorsoventrally and appears ovate (Davis 1956). Both sexes have a similar shape, though the male is smaller in size and tapered at the end (Gunn 1933). The first eight segments of the male bed bug abdomen are the pregenital segments (Davis 1956). Only the first seven segments of the abdomen on the female bed bug are the pregenital segments.

The first abdominal segment of the bed bug has a small triangular plate on it (Usinger 1966) and is considerably reduced (Christophers and Cragg 1922). The second abdominal segment has short broad plates on either end distally. It also consists of a larger thin plate in the center. The third abdominal segment has two very small and thin plates centered anteriorly. These three segments with their distinct partially sclerotized segments can be used to distinguish nymphs from adults (Usinger 1966).

The fourth through ninth abdominal segments are sclerotized completely dorsally (Usinger 1966). The third to fifth segments have darkened areas along the median of the bed bug, which are the dorsal scent glands. On the sixth segment there is a pair of spots in the same position as the dorsal scent glands, though these are smaller (Usinger 1966). There are dorsal scent glands spiracles on each segment except the remaining genital segments (Davis 1956). On the female bed bug's fifth abdominal segment is a paragenital sinus ecto-spermalage (Usinger 1966) also called the Organ of Burlese (Davis 1956). This organ is used during copulation (Davis 1956). During copulation the male bed bug must twist around the female bed bug in order to inseminate her through the Organ of Burlese. This is known as traumatic insemination.

The male bed bug reproductive system is located on the ninth abdominal segment (Figure 4). In order for the male bed bug to mate, the ninth abdominal segment must bend sharply to the left. This bending is enabled by having the left side of the eighth segment narrower than the right side (Davis 1956). The modified paramere arises in the ninth segment and always curves to the left (Usinger 1966), sitting in a groove on the ninth segment. The male bed bug's genitalia is too modified for the normal route of reproduction with the female's traditional reproductive tract.

The female reproductive system occupies the eighth and ninth segment of the bed bug abdomen (Figure 5; Davis 1956). The first gonapophyses are joined together by a membranous cuticle (Davis 1956) and are located on the eighth abdominal segment. The second gonapophyses, on the ninth abdominal segment, are reduced lobes beneath the first gonapophyses with their bases forming the sclerotized rami, the leaves connecting gonopophyses to gonocoxites and the body wall (Usinger 1966). Though the bed bug female reproductive system is fully functional, bed bugs mate through traumatic insemination instead of through the typical reproductive tract.

**Reproduction.** Cimicids have a unique form of mating called traumatic insemination. Traumatic insemination begins with the male bed bug puncturing the female body wall with his paramere, causing an integumental wound. During copulation, sperm is injected into the female bed bug abdomen outside the normal reproductive tract (Usinger 1966).

Because male bed bugs puncture the female bed bug in the abdomen, female bed bugs have developed the Organ of Burlese to help aid in copulation and survival (Davis 1956). The Organ of Burlese is located on the sixth abdominal segment of the female bed bug (Davis 1956), slightly behind the anterior margin. The bed bug male genitalia have been modified to puncture the Organ of Burlese.

During copulation the male bed bug must climb on the female bed bug's back in order to twist his body into the correct position to insert his paramere into the Organ of Burlese. Once the male bed bug is situated on the female bed bug's back, he bends his abdomen around the female bed bug's left side and inserts his paramere into the Organ of Burlese (Davis 1956). Once copulation begins, the paramere enters the Organ of Burlese and injects the sperm inside the female in one mass (Usinger 1966). Copulation normally lasts between 1 to 5 minutes, but can last up to 30 minutes in some cases. After three to four hours, sperm diffuse from the mass and make their way to the periphery of the Organ of Burlese (Davis 1956). When most of the spermatozoa have accumulated on the walls, the Organ of Burlese ruptures and the spermatozoa flow out into the body cavity towards the seminal conceptacles. Some of the spermatozoa penetrate the oviduct directly and go directly to the ovaries (Usinger 1966). Once inside the seminal conceptacles, the sperm travels into the genital chamber and then up the mesodermal oviducts to the ovaries fertilizing the eggs (Usinger 1966). Three to six days after mating, eggs

are deposited onto cracks and crevices near the bed bug harborage (Hase 1917) either singly or in clusters.

**Life History.** Adult bed bugs can survive up to 18 months without feeding if a host is not present and the bed bugs have had at least one blood meal (Bacot 1914). The adult bed bug is a broadly flattened, ovoid, insect with greatly reduced wings, roughly ¼ inch long (Schuh and Slater 1995). Adult bed bugs mate through traumatic insemination because of specialized reproductive organs. Bed bug mating occurs when the male mounts the back of the female, pushes his abdomen around the female's abdomen, and pushes his paramere into the female's spermalage (Carayon 1964). Mating usually lasts for several minutes but can last up to 30 minutes in some cases (Rivnay 1933). Mating will not occur unless the males have fed within the previous 2 weeks of mating and the females within the previous few days (Mellanby 1935). Bed bug eggs are laid on a substrate 3-6 days after mating (Hase 1917). The female bed bug lays approximately 200 eggs during her life, laying 1 to 12 eggs per day (Krueger 2000).

Bed bug eggs are about 1 mm in length, white, elongate, and slightly bow shaped. When laid, the eggs are coated with sticky cement that dries quickly after the egg is deposited, causing the eggs to adhere to the object on which they were deposited (Usinger 1966). Eggs are typically laid in cracks and crevices near a harborage site. Eggs normally take 6-10 days to hatch, though temperature variation may alter hatch time (Johnson 1940). As a first instar bed bug emerges from the egg, anti-peristaltic movements of the gut drive fluid into the head, deploying hatching spines to dislodge the egg cap (Sikes and Wigglesworth 1931). After the bed bug hatches, the nymph will attempt to find a host to feed on.

First instar bed bugs can feed within 24 hours after hatching but can survive 83.7 days on average without feeding if no host is present (Kemper 1930). Ordinarily, nymphal bed bugs will

feed once per instar, but if insufficient blood meals are taken, secondary blood meals can be necessary to reach further development (Kemper 1936). Bed bugs have five nymphal instars, each requiring a blood meal of sufficient nutrition to complete development. Each nymphal stage can feed within 24 hours after molting (Titschack 1930) and bed bugs are stimulated to feed at weekly intervals (Kemper 1936). At higher temperatures (27°C), feeding interval time can be reduced to every 3 days (Kemper 1936). If adverse conditions are present, nymphal development can take up to 156 days (Girault 1912) or longer. After 5<sup>th</sup> instar bed bugs have molted into adults, they try to feed immediately.

**Bed Bug Modes of Infestation and Harborage.** *Bed bug modes of infestation.* Bed bugs can be moved from home to home by various methods. Bed bugs can travel freely from apartment to neighboring apartment on their own via wall void or plumbing connections (Gunn 1933). They can also be moved building wide by human activity (Potter 2006). Because bed bugs are ectoparasites, they feed on humans but do not live on the host (Price 1977). Therefore, bed bugs are typically transported in their host's belongings rather than on the host's body. Bed bugs can hitch hike into a home on luggage, clothing, baggage, or in laundry bags (Harlan 2006a, Kells 2006a, Usinger 1966). Old furniture that has been stored or bought at an infested location may bring bed bugs into a home as well (Gunn 1933). Transportation venues and establishments may function as temporary harborages where bed bugs can be picked up and disbursed to numerous new locations. Usinger (1966) wrote of being bitten by a bed bug on the hand while traveling on a bus in Georgia. Other temporary nesting sites bed bugs can infest are hotels, movie theatres, bus stations, airports, and taxi stands (Kells 2006a). Once bed bugs are picked up from one of these sites, they can be easily brought into a home (Kells 2006a).



*Bed bug harborage:* Bed bugs are thigmotrophic insects (Usinger 1966), seeking shelter in cracks and crevices with rough substrates (wood, cardboard, wallpaper) in a dwelling (Gunn 1933). Bed bugs can live anywhere in a dwelling, residing in the cracks and crevices of bedding, furniture, walls, floors (Hwang et al. 2005), behind pictures, wallpaper, light switches, door and window frames, baseboards, and wall panels. Bed bugs prefer dark locations (Hase 1917), with little air flow (Kemper 1936), and minimal disturbance (Cooper 2006). These harborages are not evenly distributed throughout the host's dwelling (Usinger 1966). So bed bugs are often found aggregating in preferred harborages where clumps of bed bugs are clustered together around bed bug frass, eggs, and exuvia (Kemper 1936). These aggregations are a result of aggregation pheromones (Kemper 1936). Bed bugs return to these harborages after taking a blood meal and remain there until digestion and/or molting is completed (Kemper 1936).

**Host Detection.** The means for host detection is not fully understood. However, the primary stimulus for bed bugs to leave their harborage is hunger (Usinger 1966). Heat is definitely a factor for host detection, although there has been controversy as to how far a bed bug can detect heat (Usinger 1966). There has been evidence that bed bugs can detect a host from up to 150 cm away (Marx 1955). However, others have concluded that bed bugs only detect a person if they are 3-4 cm away (Hase 1917, Rivnay 1932, Kemper 1932). Usinger (1933) reported that bed bugs could not detect a host beyond 5 ft and doubts they could detect a host beyond a few inches.

Bed bugs are attracted to the highest temperature heat source in their vicinity (Usinger 1966). In laboratory tests, bed bugs repeatedly chose a higher temperature in a T-tube evaluations where humans (37 °C) and rabbits (39.6 °C) were tested against higher temperature

blanks (49.6 °C and 59 °C) (Usinger 1966). In these tests, bed bugs chose to go to the highest temperature source in each trial.

Odor from blood or perspiration alone does not appear to be an attractant to bed bugs (Rivnay 1932). However, bed bugs are attracted to CO<sub>2</sub> when accompanied by heat, as in the form of breath (Hase 1917).

Another possible method of bed bug host detection is the use of a pheromone trail. Bed bugs have been observed laying trails of fecal spots by dragging the abdomen along a surface (Hase 1917). However, Kemper (1936) found that bed bugs will take considerable detours following these pheromone trails when a host is located at a distance over 20 m.

### **Medical Importance of Bed Bugs**

With bed bug infestations making a comeback around the globe, the potential health issues associated with bed bugs becomes a concern (Boase 2004). Elias Metschnikoff (1887) was the first to suggest bed bugs had the possibility of transmitting diseases. But Nuttall (1900) did not show any conclusive evidence to suggest that bed bugs had the potential to transmit different diseases. Although bed bugs have never been implicated as vectors of human disease, bed bugs are suspected carriers of leprosy, oriental sore, Q-fever, brucellosis (Dolling 1991), Chaga's Disease (Pipkin 1969), filariasis, mansonelliasis, kala-azar, espundia, septicemia, anthrax, pneumonia type 2, tularemia, brucellosis, paratyphoid fever, plague, rocky mountain spotted fever, epidemic typhus, murine typhus, relapsing fever (*B. recurrentis*, *B. duttoni*, *Spirochaeta merionesi*), infectious jaundice, poliomyelitis, yellow fever, smallpox, and lymphocytic choriomeningitis (Burton 1963). Human Immunodeficiency Virus (HIV) can survive in bed bugs for up to an hour after ingestion, but there is no epidemiological evidence

displaying that HIV can be transmitted from feeding on a person (Lyons et al. 1986, Jupp and Lyons 1987, Webb et al. 1989). There is also evidence that the Hepatitis B virus may be mechanically transmitted to humans in bed bug feces or when bed bugs are crushed during feeding (Blow et al. 2001). However, Hepatitis C virus was unable to persist in bed bugs (Silverman et al. 2001). Nonetheless, there has not been any substantial evidence of disease transmission from bed bugs to humans (Craig and Faust 1970). Unfortunately, disease transmission is not the only concern when it comes to bed bug bites.

Bed bug bites have a wide variety of reactions on a person by person basis. Feeding on the blood of a host, bed bugs puncture the host's skin with their piercing-sucking mouthparts and can cause a reaction on the individual as they withdraw blood and liquefy epidermal tissue (Elston and Stockwell 2000). Some individuals have no reaction (Kemper 1936) and others have a wheal for a reaction (Hase 1917). Reactions depend on an individual's immunocompetence and previous exposure to bed bug bites (Sansom et al 1992). These reactions ranging from wheals, erthema, vesicle formation (Harves and Millikan 1975), lesions, erythematous papules (Kettle 1995) and bullous eruptions (Tharakaram 1999, Liebold et al. 2003) are caused by an injection of substances such as hyaluronidase, protease, and kinins (Harves and Millikan 1975). Lumps that may occur are an allergic reaction (Hect 1930, Potter 1997).

Bed bugs have a number of other factors that need to be considered along with the reactions to their bites. Secondary bacterial infections may occur from vigorous scratching and excoriation (Burnett et al. 1986). A rash may also occur from the bites (Liebold et al. 2003). Bed bugs can cause nervousness, lethargy, pallor, diarrhea, (Whitson et al. 2001) and eventually iron deficiencies (Venkatachalam and Belavadi 1962). There have been cases of iron deficiency caused by excessive feeding of bed bugs on infants in India (Venkatachalam and Belavadi 1962).

An iron deficiency was also recorded by Usinger (1966) in which case his hemoglobin levels decreased from 14.5g per 10 cc of blood to 11.5g over a 6 year period (1958-1964). It is also suspected that bed bugs may also be a contributing factor to asthma in people living with large populations of bed bugs (Fu et al. 1995). Asthma occurs when an individual has a hypersensitive reaction to bed bug bites (Huntley 1999). Symptoms for bed bug bites can vary widely, making diagnosis difficult.

Health care providers are ignorant about bed bug symptoms (Scarupa 2006) resulting in misdiagnosis across the nation. Because individuals have different reactions to bed bug bites, diagnosing the bite reaction can be difficult. Since bed bug bites are not typically felt, the host becomes aware of the bite when it begins to itch, burn, swell, or become enflamed (Liebold et al. 2003). Bed bug bites can leave doctors wondering about what diagnosis to give to their patients. Bed bug reactions may look like a drug reaction, allergic contact dermatitis, atopic dermatitis, Gianotti-Crosti syndrome, pityriasis lichenoides et varioliformis acuta, ecthyma, scabies infestation, or dermatitis herpetiformis (Tharakam 1999, Jordan and Schneider 1997). With so many other diseases having symptoms similar to bed bug bites, diagnosing bed bug bites correctly can be difficult. However, bed bugs should be considered if an individual has any insect bites, especially if the bites cannot be explained (Cooper 2006). If bed bugs are suspected, then a thorough inspection of the home is needed to verify if they are a possibility.

### **Bed Bug Control**

**Monitoring and Visual Inspections.** Bed bugs are cryptic by nature, so initially locating an infestation, especially if numbers are low, can be challenging. Bed bugs are thigmotrophic (Usinger 1966), reacting negatively to light (Usinger 1966), and avoid air currents

(Kemper 1936). Therefore, finding a way to attract or monitor for bed bugs is difficult. There have been studies evaluating bed bug attraction to human volatiles such as carbon dioxide, perspiration, and various other scents but with little success. Heat is the only consistent bed bug attractant ever documented (~32° C; Usinger 1966).

A monitor for bed bugs could be advantageous for determining if there is an insipient bed bug infestation or if a particular treatment regimen has been successful. However, there is no known device to attract or trap bed bugs (Harlan 2006a). Sticky traps, commonly used for monitoring a wide variety of insect pests, have yet to be proven effective for locating bed bugs (Cooper and Harlan 2004). However, historic literature cites “Demon” cockroach traps and corrugated cardboard as effective methods for collecting bed bugs in a field study (Mellanby 1939).

Currently monitoring devices are not reliable, so pest control professionals must rely on visual inspections to determine if there is an infestation, or to determine the level of infestation at a particular location (Harlan 2006a, Potter 2006). Because there is a lack of monitoring tools, it is difficult to establish an early bed bug detection program, or to evaluate how an ongoing bed bug management effort is progressing. Bed bugs can sequester themselves in small cracks and crevices, as well as inaccessible areas, making the inspection for bed bug evidence crucial (Cooper and Harlan 2004). If the only evidence of an early infestation is a single exuvia, a few eggs, or fecal specks within an apartment these subtle clues may be very difficult to detect (Potter 2006).

One important step to determine how long the dwelling has been infested, is to find out if the tenant has traveled recently (in the last year) or has received any used furniture (Cooper and Harlan 2004). Determining when an infestation got started can give valuable clues as to the level

of the infestation. If the infestation has been present for a few months, bed bugs will have established many harborage locations. The effective management of the infestation is completely reliant on the pest control operator's ability to locate all harborages occupied by bed bugs (Cooper and Harlan 2004, Harlan 2006a). Every possible harborage location should be viewed as a potential bed bug foci.

Bed bugs require an environment where the temperature and humidity are within the range preferred by humans. Bed bugs also need a place to rest and a host, so the host's primary sleeping area should be considered for inspection first (Kells 2006a). Not only should the inspection be thorough, looking in every possible harborage location, the inspection needs to be methodical with each room being inspected systematically (Doggett et al. 2004, Harlan 2006a, Kells 2006b). The written inspection report then needs to be detailed, including a rough estimate of the population, the rooms that are infested, and all harborage locations (Harlan 2006a).

Before an inspection begins, a "clean area" needs to be established in the room (Kells 2006b). This clean area will serve as the treatment location for all belongings in a room. Furniture and other belongings will be moved into the clean area and treated. As the initial clean area fills up, other areas of the room will become available for inspection and treatment. Inspections need to start with the bed components. All bed and furniture should be disassembled so cracks and crevices can be inspected (Kells 2006b). While inspecting, flushing agents can be used to flush out bed bugs and expose harborage locations (Cooper and Harlan 2004, Harlan 2006b). Bed bug inspections and treatments are very time consuming. One current recommendation is to have two pest control operators working together to completely inspect and treat a location. It is generally accepted that a thorough inspection and treatment may take ~1.5 hours or more.

Because bed bug inspections are inherently difficult, time consuming, and prone to human error, dogs are being trained as bed bug inspectors, and have been used to detect a wide variety of hidden materials (drugs, bombs, mold). Dogs are also currently being used to detect subterranean termite infestations (Brooks et al. 2003). Dogs have also shown high levels (97%) of accuracy for detecting carpenter ants (Brooks et al. 2003). Recently, there has been interest in using dogs for detecting bed bugs. Latimer (2006), a master K9 trainer, has been testing dogs for detecting bed bugs, and claims greater than 90% accuracy. If dogs are able to detect a bed bug infestation, especially in inaccessible locations (behind baseboards, inside of furniture, etc.) then they would be a great asset for bed bug management programs.

**Treatment Methods.** *Non-chemical control measures.* There are a variety of non-chemical bed bug control measures which are extremely important for bed bug management. The majority of these methods are directly related to improving the sanitation of the infested location. For example, vacuuming can be used during an inspection, or for control efforts, to quickly capture and contain bed bugs (Kells 2006b). Vacuuming can collect bed bugs which are exposed along tufts of mattresses and box springs. Vacuums can also collect bed bugs along the edges of carpeting, in cracks of wood floors, behind wall paper, and a variety of other locations. However, vacuums cannot penetrate deep inside upholstered furniture nor can they dislodge bed bug eggs which are typically cemented to the substrate.

Different types of thermal treatments can be used for bed bug control. Dry heat, freezing, and steam treatments are options that can aid in a bed bug treatment programs. Heating the core of a couch or mattress to 120° F (45° C) which is the bed bug's thermal death point will kill bed bugs in that piece of furniture (Cooper and Harlan 2004, Kells 2006b). Freezing is another option for bed bug control. Holding a piece of furniture at 23° F (-5° C) for at least 5 days will

kill bed bugs (Kells 2006b). When using a cold thermal treatment, the time of exposure is reduced as the exposure temperature is decreased. If you want to flash freeze bed bugs, a target temperature capable of killing bed bug eggs is -15° F (-26° C: Kells 2006b). Another option for a thermal treatment is steam. If steam is applied correctly, it is capable of killing bed bugs eggs and all stages of exposed bed bugs including those bed bugs hidden inside a mattress (Cooper and Harlan 2004, Kells 2006b). Unfortunately, steam treatments are very time consuming and labor intensive.

Mattress covers are an easy to use treatment that can aid in bed bug control. Mattress covers can serve to keep bed bugs contained inside the infested cover, eliminating the need to throw the mattress away (Kells 2006b). Mattress covers can also be used to prevent bed bugs from infesting an un-infested mattress (Kells 2006b). If a chemical treatment is applied to a mattress, mattress covers can be used to contain the pesticide product and reduce human exposure (Kells 2006b).

Physical barriers can aid in controlling a bed bug infestation. Because bed bugs can readily infest wall voids and will travel along pipes or other utilities entering adjacent rooms or apartments, the gaps around all pipe work and other utilities need to be sealed where the pipes enter the living space of the dwelling (Cooper and Harlan 2004). All cracks and crevices need to be sealed (caulked) in order to eliminate bed bug access and harborage locations (Cooper and Harlan 2004). Less obvious barriers include pulling the bed away from the wall to eliminate a route for bed bugs to gain access to a host. Greasing the bed legs with petroleum jelly, or setting the legs inside dishes filled with soapy water, is another way to eliminate bed bug access to the sleeping host (Olkowski et al. 1991).



Poor sanitation can contribute to a thriving bed bug infestation. Cluttered conditions provide numerous bed bug harborages, stacks of books, papers, boxes, piles of laundry and other belongings should be eliminated (Cooper and Harlan 2004). Besides creating excellent harborages for bed bugs, cluttered conditions also make it difficult for pest control operators to inspect and treat an infestation. Improving sanitation will make the environment less hospitable for bed bugs and increase the ability to treat effectively.

While non-chemical control methods are extremely valuable and necessary when attempting to manage a bed bug infestation, none of these non-chemical control measures will be successful if used alone (Kells 2006b). Therefore, chemical control methods should be combined with non-chemical control methods as part of an integrated bed bug program.

*Chemical controls.* Currently, insecticide treatments are necessary for both short-term and long-term bed bug management (Cooper and Harlan 2004). All potential harborage sites need to be treated with labeled insecticides (Harlan 2006b). Dusts, microencapsulated materials, and wettable powders are the most effective formulations currently available for bed bug control (Cooper and Harlan 2004).

Residual dusts or liquids are applied to cracks and crevices in order to provide control of harboring bed bugs. These long lasting products will also increase the likelihood of bed bugs encountering a treated surface when searching for the host. Liquid and dust formulations of pyrethroids are currently the most widely used insecticides for bed bug control. Currently, the vast majority of bed bug products labeled for bed bug control are pyrethroids. However, there has been some concern that pyrethroids are repellent to bed bugs (Cooper and Harlan 2004). Pyrethroids are known to be repellant to cockroaches, (Ebeling et al. 1966) termites (Smith 1979), and ants (Knight and Rust 1990). Another concern is the potential for bed bugs to be

resistant to pyrethroids, which has been a factor for bed bug control with other insecticides in the past (Usinger 1966, Ebeling 1975, Cooper and Harlan 2004).

Newer products, which are non-repellent, are also available for bed bug control (Harlan 2006b). These newer chemistries have the advantage of both not being repellent to bed bugs and having no documentation of bed bug resistance (Cooper and Harlan 2004). These newer chemistries have novel active ingredients such as chlorfenapyr, which is a pyrrole. Chlorfenapyr functions as a stomach toxicant where the insecticide metabolite produces the toxic effects. Another active ingredient recently labeled for bed bugs is hydroprene. Hydroprene is a juvenile hormone analog which is designed to cause sterility in insects. Other novel products include non-insecticidal formulations such as N.I.C. 325 (active ingredients: limestone and corn gluten meal) and Steri-fab (active ingredient: isopropyl alcohol). These products are labeled for bed bug control and can be used as mattress treatments.

No matter which insecticides or combination of insecticides are used for bed bug control, follow-up inspections and treatments are essential. Treating the infested room is obvious, but treating adjacent rooms is also necessary for bed bug elimination. Bed bugs can move from one location to another readily, so it is important that adjacent rooms or apartments in multi-unit facilities are inspected and possibly treated. Cooper and Harlan (2004) recommend that in multi-unit housing facilities, that apartments adjacent to an infested unit also be inspected and or treated for bed bugs. This includes the apartment above, below, and on either side of the infested unit. In addition, it is recommended that wall voids between infested rooms or apartments be treated with a labeled dust formulation. It is not uncommon to have an “eliminated” bed bug infestation reappear after several months (Cooper and Harlan 2004). Therefore, a single pest

control service is unlikely to be successful because bed bugs are cryptic and able to live for long periods without feeding (Cooper and Harlan 2004, Usinger 1966).

The inspection and treatment methods presented above are the current recommendations that are being disseminated throughout the pest control industry via trade magazines, conferences, and word of mouth. However, very few of these treatment methods have been empirically tested. The goal of the research project presented in the following pages was to evaluate several of these bed bug treatment methods so that proper recommendations could be provided to the pest control industry and the general public.

Figure 2.1. Dorsal view of nymphal bed bug anatomy. page 30

Figure 2.2. Dorsal View of an adult bed bug anatomy. page 31

Figure 2.3. Ventral View of an adult bed bug anatomy. page 32

Figure 2.4. Ventral view of adult bed bug thorax. page 33

Figure 2.5. Genitalia of a male and female bed bug. page 34

\* All images were scanned from the Monograph of Cimicidae (Usinger 1966) with Permission from the Thomas Say foundation.

**Figure 2.1. Dorsal View of Nymphal bed bug**  
 scanned from the Monograph of Cimicidae (Usinger 1966) with Permission  
 from the Thomas Say foundation.

MORPHOLOGY

51

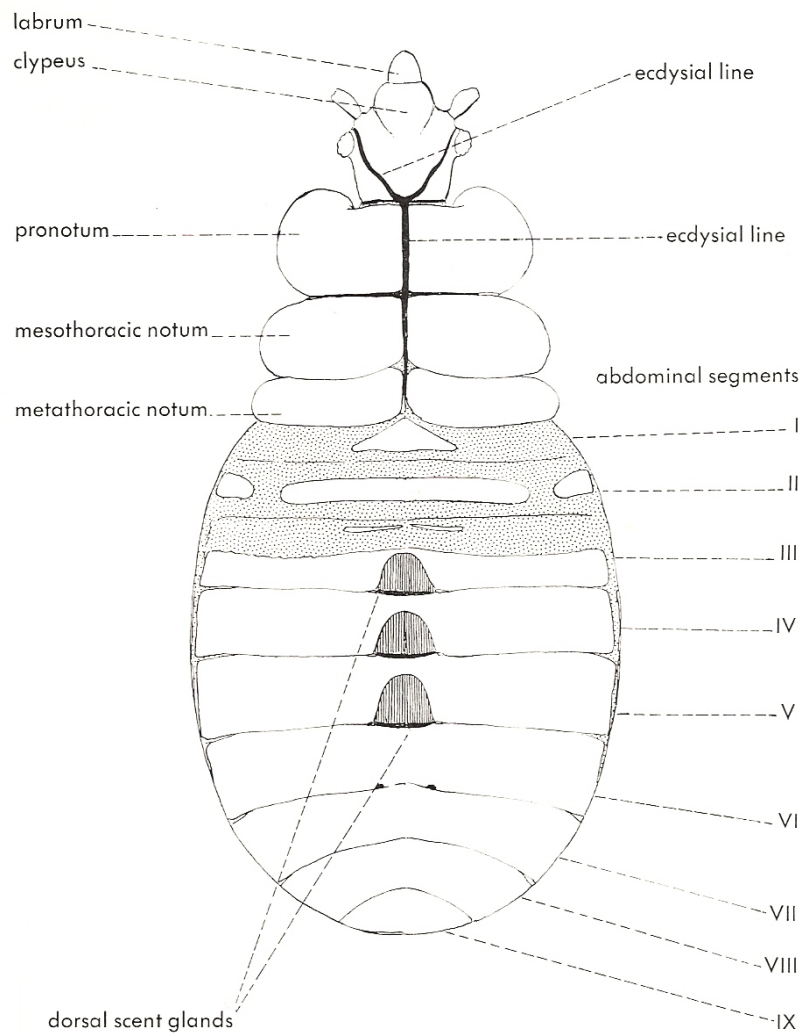


FIG. 6-1.—Last-stage nymph, *Cimex lectularius*, dorsal view (Ferris 1957a) .

**Figure 2.2.** Dorsal View of an adult bed bug anatomy  
 scanned from the Monograph of Cimicidae (Usinger 1966) with Permission  
 from the Thomas Say foundation.

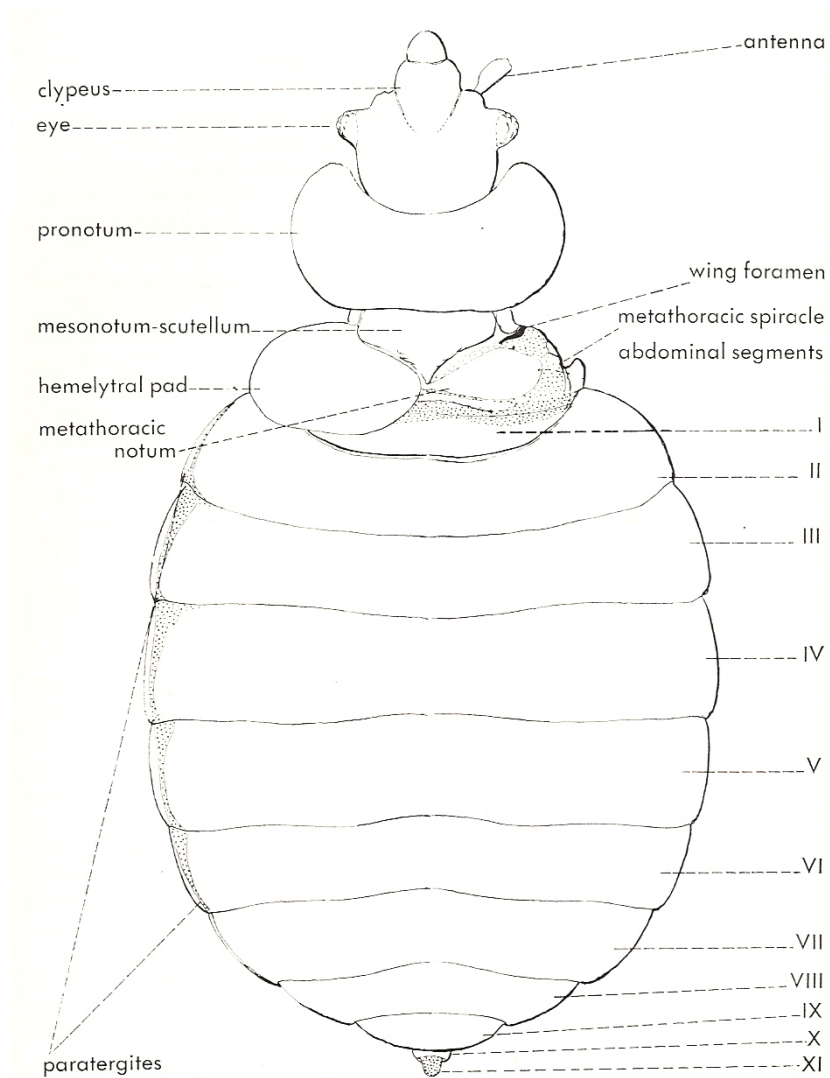


FIG. 6-4.—Adult female, *Cimex lectularius*, dorsal view (Ferris 1957a) .

**Figure 2.3. Ventral View of an adult bed bug anatomy**  
 scanned from the Monograph of Cimicidae (Usinger 1966) with Permission  
 from the Thomas Say foundation.

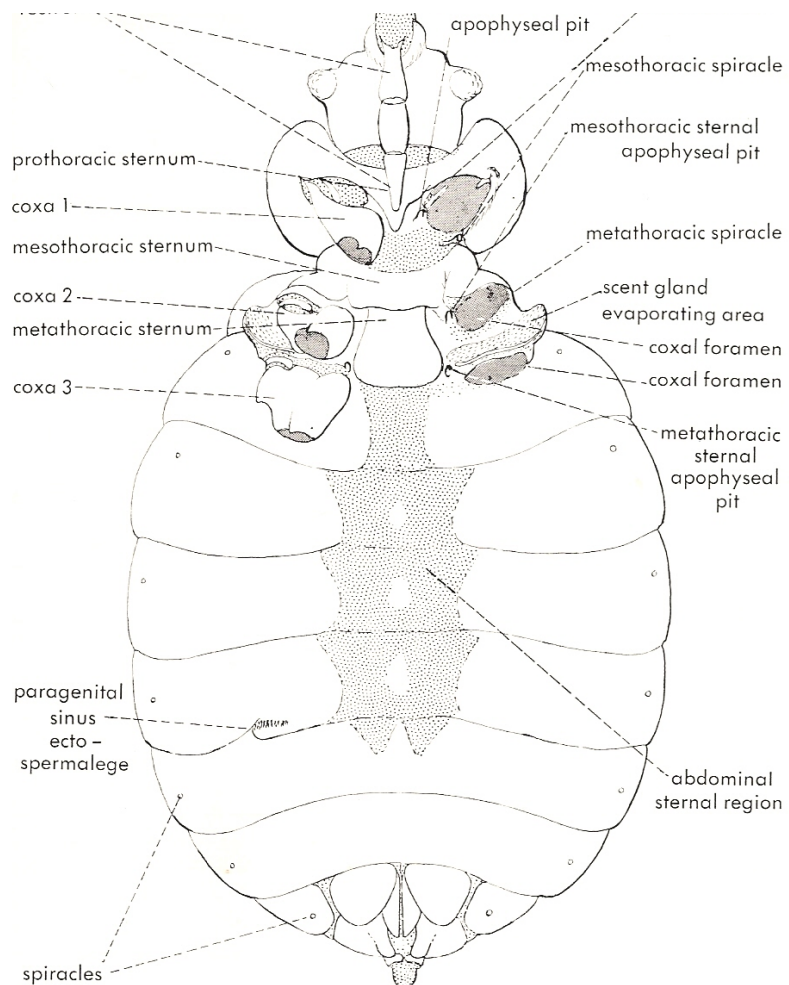


FIG. 6-5.—Adult female, *Cimex lectularius*, ventral view (Ferris 1957a) .

**Figure 2.4. Ventral View of adult bed bug thorax**  
 scanned from the Monograph of Cimicidae (Usinger 1966) with Permission  
 from the Thomas Say foundation.

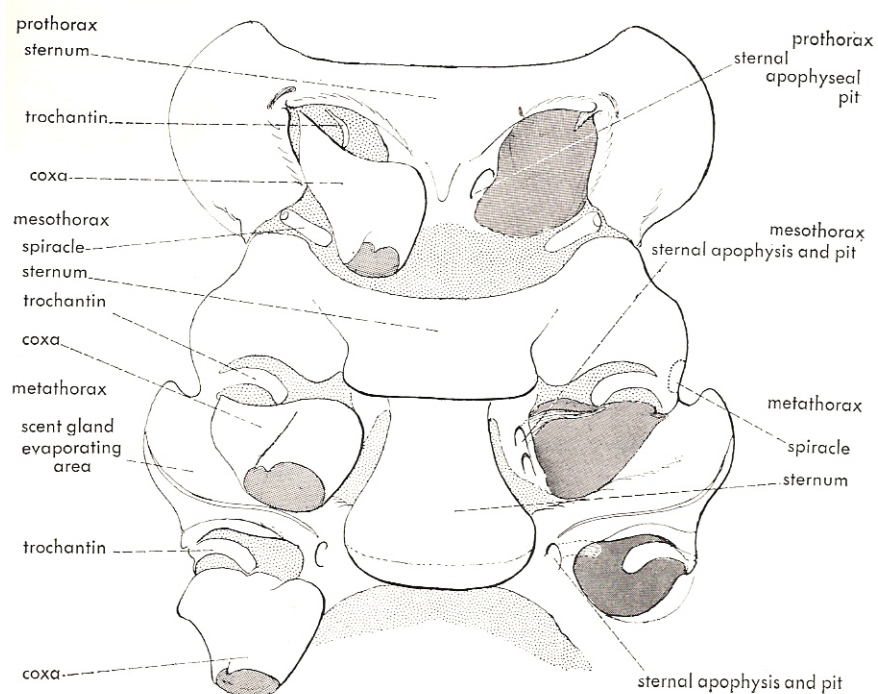


FIG. 6-6.—Adult *Cimex lectularius*, ventral aspect of thorax (Ferris 1957a).



**Figure 2.5.** Genitalia of a male and female bed bugs scanned from the Monograph of Cimicidae (Usinger 1966) with Permission from the Thomas Say foundation.

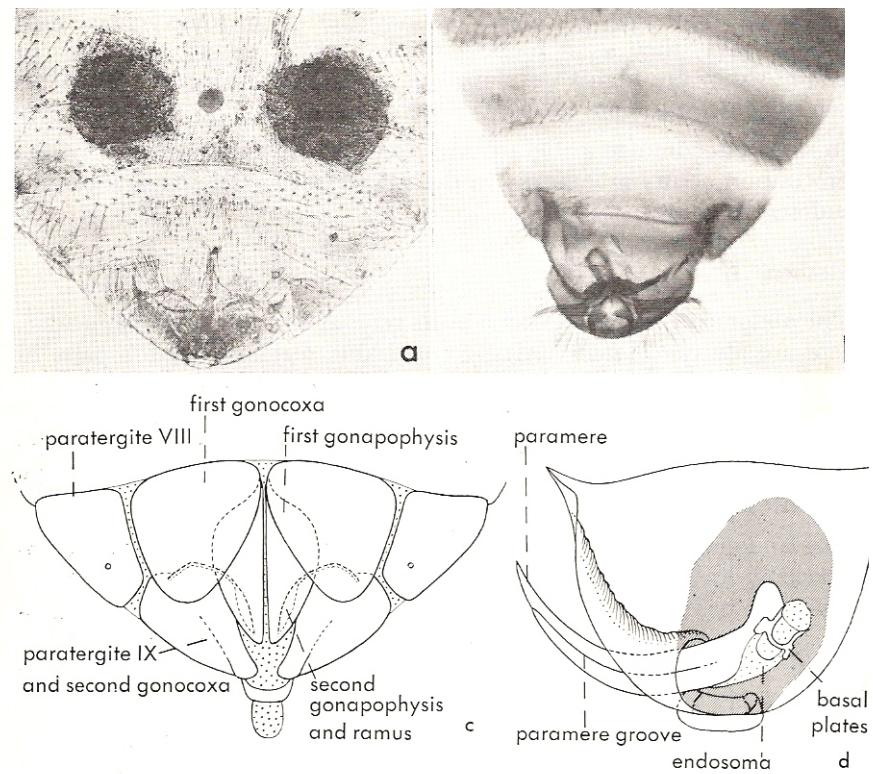


FIG. 6-8.—a, *Oeciacus hirundinis*, female with two spermaleges (Carayon); b, *Cimex hemipterus*, male with two parameres; c, *Cimex lectularius*, female genitalia, ventral view; d, *Cimex lectularius*, male genitalia, dorsal view.

# **Chapter Three**

## **Efficacy and Repellency of Insecticides Labeled for Bed Bug Control**

### **Introduction**

At the beginning of the 20th century, the bed bug was a common household pest in the U.S. and throughout the world (Ebeling 1975). Bed bugs were frequently encountered in quality hotels and motels as well as less respectable establishments and were easily transported into travelers' home via their clothes, vehicles, or luggage. Once inside the home, bed bugs can find harborage in furniture, cracks and crevices in the floor or walls, behind wallpaper, or under carpeting (Krueger 2000).

The importance of the bed bug as a household pest began to diminish in the 1940s and '50s with the use of pyrethrum insecticides and DDT (Ebeling 1975). In the latter half of the 20th century the bed bug was almost eradicated in the United States, with only a few persistent populations surviving in locations where living conditions were primitive or unsanitary (Ebeling 1975).

However, in the early 1990s pest management professionals began to see an increase in bed bug infestations across the nation (Krueger 2000). The reason for this increase is not known, but increased international travel (Potter 1997), a reduction in the use of residual insecticides indoors, and insecticide resistance may be contributing factors (Potter 2005). Although international travel may be considered a factor in the bed bug increase, international travel has been taking place for several decades and the bed bugs have only recently (1990s) become recognized as a widespread problem. The second factor, reduction in residual pesticide use indoors, may be a realistic contributor. Concerns about human exposure risk have greatly reduced routine interior applications of spray insecticide in favor of integrated pest management (IPM), where insecticides are applied only on an as-needed basis. The elimination of baseboard

spraying would allow incipient bed bug infestations to proliferate unchecked. In addition, the use of insecticide baits as part of IPM is preferred over liquid insecticide treatments. However, insecticide baits do not affect blood sucking parasites. The third factor that could be responsible for bed bug resurgence is widespread resistance to insecticides within the bed bug population. Bed bug populations in developing nations and the U.S. have been treated repeatedly with a variety of insecticide products. It is quite possible that these populations are now resistant to many insecticides used for their control.

Bed bug resistance to a number of insecticides has been well documented. These insecticides include DDT, methoxychlor, dieldrin, aldrin and other organochlorine compounds (Busvine 1980), carbaryl, endrin, gamma BHC (Radwan et al. 1972), malathion and other organophosphates, and sodium fluoride (Feroz 1974). Many of these older chemistries have been banned for use in the United States by the U.S. Environmental Protection Agency and are no longer available for bed bug treatment.

Because bed bugs have not been a problem in the U.S. for over 40 years, few of the currently available insecticide products are labeled for bed bug control. Those products labeled for bed bug treatment are typically either natural pyrethrins or pyrethroid products. However, the efficacy of these products has not been empirically determined. Because the active ingredients in these products are from the same chemical class, resistance to these products could easily develop. In addition, most pyrethroid-type chemistry has been shown to be repellant to crawling pests such as cockroaches, (Ebeling et al. 1966) termites (Smith 1979), and ants (Knight and Rust 1990). The potential for repellency suggests that aggregations of bed bugs may scatter if they are treated with these products directly or that individual bed bugs may avoid

surfaces treated with pyrethroids. These behavioral responses would hinder pyrethroid efficacy as a bed bug control product.

There are also several non-pyrethroid products that are being widely used for bed bug control. One of these products is Phantom (chlorfenapyr 0.5%), a residual crack and crevice spray that is not specifically labeled for bed bugs, but that has a site label that allows for the indoor treatment for crawling insects. Another product is Natural Insect Control dust (N.I.C. 325, limestone, and corn gluten meal) which is a desiccant dust labeled for mattress and crack and crevice treatment. Although pyrethroids, chlorfenapyr, and N.I.C. 325 are currently being used by the pest management industry, there is no published data to indicate that any of these products kill bed bugs. The purpose of this study was to evaluate the efficacy and potential repellency of several insecticide formulations currently being used for bed bug control.

## **Materials and Methods**

**Bed Bug Rearing.** A susceptible strain of bed bugs, *Cimex lectularius* (Linnaeus), was acquired from Dr. Harold Harlan of the National Pest Management Association in February 2005. Dr. Harlan maintained this colony of bed bugs for over 32 years (since 1973), feeding them on himself. The susceptible strain bed bugs were contained in glass jars (wide mouth Mason, 360 ml) fitted with a standard twist-off metal ring lid over a plastic mesh top to facilitate bed bug feeding. Inside the jar, cardboard (4 cm x 10 cm) had been folded accordion style and stood on end so that bed bugs were able to crawl up the cardboard and feed through the mesh top.

Bed bug colonies have been reared in the Dodson Urban Pest Management Laboratory and maintained similarly to the method described above. Bed bugs were fed by a human host once a week. Feeding took place by having a host place their forearm against the mesh top of the

colony rearing jars. Each jar of bed bugs was allowed to feed on the host for 30 minutes. Bed bug feeding was conducted as approved by the Virginia Tech Institutional Review Board (IRB #06-165). Between feedings, bed bugs in rearing jars were stored in a closed incubator at ~27°C, ~60% RH, and a photoperiod of 12:12 L:D. In February 2006, a field strain of bed bugs was collected from an apartment complex (Buckingham Village) in Arlington, VA. The rearing and feeding methods used to maintain the field strain bed bugs were the same as those described for the susceptible strain.

**Insecticide Efficacy Bioassay.** Insecticide efficacy was determined by calculating the  $LT_{50}$  values for the susceptible and field strain bed bugs exposed to insecticide products. The  $LT_{50}$  values for each of the test insecticides were calculated using the standard procedure of confining bed bugs on insecticide treated surfaces and recording mortality at regular time intervals. The liquid insecticides tested using susceptible strain bed bugs were lambda-cyhalothrin (Demand CS, 0.03%; Syngenta, Greensboro, NC), cyfluthrin (Tempo SC Ultra, 0.05%, Bayer CropScience LP, Montvale, NJ), bifenthrin (Talstar One, 0.02%; FMC Corp., Philadelphia, PA), deltamethrin (Suspend SC, 0.06%; Bayer Environmental Science, Montvale, NJ), permethrin (Dragnet SFR, 0.5%; FMC Corporation, Philadelphia, PA), and chlorfenapyr (Phantom, 0.5%; BASF, Research Triangle Park, NC). N.I.C. 325 (99.5% lime stone and 0.5% corn gluten meal; AMC, Fort Collins, CO) a dust formulation of lime stone, was also tested. Several of the pyrethroid products were also tested using the field strain bed bugs. These pyrethroids were lambda-cyhalothrin, cyfluthrin, bifenthrin, and deltamethrin.

Five days prior to testing, groups of 10 adult bed bugs (mixed sex: 50% male, 50% female) were fed to repletion, then transferred from rearing jars into a closed Petri dish that had been treated on the interior sides with fluon. For testing liquid insecticides, hardboard panels

(4x4 cm sq) were treated with products at the label rate to the point of runoff and allowed to dry for 24 h. Control panels were treated with water only. After the panels were air dried (24 h), the Petri dishes were opened and inverted onto the treated hardboard surfaces so that the insects were confined inside the dishes while in direct contact with the treated surface. Because N.I.C. 325 is a dust formulation labeled for mattress treatment, fabric was used as the test substrate. Fabric was cut into 4 x 4 cm squares and weighed. Fabric pieces were dredged through dust contained in a glass bowl until the fabric surface was covered. Dusted fabric pieces were weighed again to determine the amount of product applied on each piece of fabric (average  $109.5 \text{ mg} \pm 0.74 \text{ mg}$ ). Fabric pieces were placed on top of the hardboard panels and the bed bugs confined in Petri-dishes were inverted onto the treated fabric as described above. The number of dead bed bugs on the treated substrates was recorded at regular time intervals (every 10 minutes for the first hour, every 15 minutes for the second hour, at 4 hr, 8 hr, 12, 24 hr, and every 24 hr thereafter). Each insecticide bioassay had 5 replications.

**Insecticide Repellency Bioassay.** Arenas used in the repellency bioassays consisted of a plastic display box (6L; 10 cm height x 25 cm width x 30 cm length). The walls of the display box were coated around the perimeter with a mixture of baby oil and petroleum jelly (30:70) to prevent bed bug escape. Each arena contained a hermit crab heater (Item # HC-30, 38°C, Zoo-Med, San Luis Obispo, CA 93401) as an attractant (Figure 1).

*Non-choice tests.* No-choice bioassays were conducted using the susceptible strain bed bugs to determine if bed bugs would contact insecticide treated panels and if mortality would result. The insecticide products tested were lambda-cyhalothrin (Demand CS, 0.03%; Syngenta, Greensboro, NC), bifenthrin (Talstar One, 0.02%; FMC Corp., Philadelphia, PA), deltamethrin (Suspend SC, 0.06%; Bayer Environmental Science, Montvale, NJ), chlorfenapyr (Phantom,

0.5%; BASF, Research Triangle Park, NC), and N.I.C. 325 (99.5% lime stone and 0.5% corn gluten meal; AMC, Fort Collins, CO).

Hardboard panels were treated on all six sides and corners with each test insecticide at the label concentration until the point of run off. Panels were allowed to air dry for 24 h prior to testing. Fabric pieces (4 x 4 cm) were used for testing the N.I.C. 325 dust formulation. Fabric pieces were weighed and dredged through the dust on both sides. After dusting, the fabric pieces were reweighed to determine the mean amount of dust applied on each substrate ( $291.2 \mu\text{g} \pm 6.0 \mu\text{g}$ ). Fabric pieces were then placed on hardboard panels. At the initiation of each test, hardboard panels or panels with fabric pieces were placed adjacent to the hermit crab heater in the test arena.

Bed bugs were fed 5 days prior to testing. After the digestion period, groups of ten adult bed bugs (mixed sex; 50% male: 50% female) were transferred from their rearing container into closed Petri dishes (60 x 15 mm). Bed bugs acclimated to the Petri dishes for 24 h. After the acclimation period the Petri dishes were then inverted into the arenas and bed bugs were released in front of, but not in contact with, the treated panel. Bed bugs were allowed to distribute themselves within the arena *ad libitum*. The number of bed bugs resting on the treated panel (all 6 legs in contact with the treated surface) and subsequent bed bug mortality were recorded every 10 minutes for 2 h. Each insecticide bioassay was replicated 5 times.

*Choice tests.* Choice tests were conducted using both the susceptible and field strain bed bugs to evaluate pyrethroid products for potential repellency. The products evaluated were lambda-cyhalothrin, cyfluthrin, bifenthrin, deltamethrin, and permethrin. Choice tests were conducted exactly as described for the non-choice insecticide bioassays, except that two panels (figure 2) were placed inside the arena, one treated with a pyrethroid and one treated with water

only (control). The number of bed bugs on either the treated or control panel and any resulting mortality, was recorded as described for the no-choice tests. Each insecticide bioassay had 5 replicates.

**Statistical Analysis.** *Insecticide efficacy bioassay.* Mortality data was corrected using Abbott's formula (1925). The  $X^2$  and the  $LT_{50}$  values for each insecticide product were calculated using Probit analysis (Robertson et al. 2003). Significant differences between treatments were indicated by the failure of the confidence intervals (95%) to overlap.

*Repellency bioassays.* In the no-choice repellency bioassay, the mean percentage of bed bugs ( $\pm$  SE) in contact with the treated panel at any of the 10 min counting periods was recorded. The mean bed bug mortality was also recorded at each of the 10 min counting periods and used to determine if the bed bugs had contacted the insecticide treated surfaces. Bed bug response, both contact and mortality, were plotted over the 2 h test period.

For each of the choice tests evaluating pyrethroid repellency, the mean percent of susceptible strain bed bugs distributed on the panels was analyzed between and within individual assays for the first 4 counting periods (10-40 minutes), after which mortality began to influence bed bug distribution. Field strain bed bug distribution on panels was compared for the first 6 counting periods (10-60 minutes) because there was no mortality. The choice test data was analyzed using the PROC MIXED Analysis of Variance (SAS Institute 2005). Values of  $P \leq 0.05$  were used to indicate significance. Dunnetts' test was used for post hoc pairwise and otherwise comparisons (SAS Institute 2005).

## Results

**Insecticide Efficacy.** The results from the  $LT_{50}$  analysis indicated that all of the pyrethroid products killed the susceptible strain bed bugs relatively quickly (Table 1). Lambda-



cyhalothrin was the fastest acting pyrethroid with an  $LT_{50}$  value of 20 min. Cyfluthrin, bifenthrin, deltamethrin, and permethrin were all significantly slower acting than lambda-cyhalothrin. Cyfluthrin was the second fastest acting pyrethroid with  $LT_{50}$  value of 44 min, followed by bifenthrin ( $LT_{50}$  value 53 min), which was significantly slower acting than cyfluthrin but significantly faster acting than deltamethrin ( $LT_{50}$  value 61 min). The slowest acting pyrethroid tested on the susceptible strain was permethrin, having an  $LT_{50}$  value of 87.6 min. This high  $LT_{50}$  value was probably due to permethrin being a third generation pyrethroid, while the rest of the pyrethroids tested were fourth generation (Ware and Whitacre 2004).

Both of the non pyrethroid products were significantly slower acting than the pyrethroids. The  $LT_{50}$  value calculated for N.I.C. 325 dust was 9 d 11 h. Chlorfenapyr had a significantly higher  $LT_{50}$  value (10 d and 9 h) than the N.I.C. 325 dust.

Overall, the field strain bed bugs were significantly less susceptible to the pyrethroid insecticide formulations than the susceptible strain bed bugs (Table 1). However, field strain bed bugs also responded differently to different insecticide formulations. The  $LT_{50}$  value calculated for field strain bed bugs exposed to lambda-cyhalothrin was 1 h 11 min. The field strain  $LT_{50}$  value for cyfluthrin was significantly greater at 12 h 10 min. The  $LT_{50}$  value for bifenthrin (3 d 16 h) was 88 times that of lambda-cyhalothrin and 7 times that of cyfluthrin. The  $LT_{50}$  value calculated for field strain bed bugs exposed to deltamethrin was significantly greater than all of the pyrethroids tested at 19 d 2 h.

**Repellency Bioassays.** *Non-choice test.* The no-choice test bioassays indicated that susceptible strain bed bugs were willing to contact the hardboard panels even when an insecticide was present. More than 50% of the bed bugs were recorded on the panels treated with lambda-cyhalothrin (Figure 3a) at the 10, 20, and 30 min counting periods. A decline in the

percentage of bed bugs on the panels started at 40 min with an onset of mortality (27%). As mortality increased, the percentage of bed bugs on the panel declined. By the end of the 2 h test period, bed bug mortality had reached 90%, indicating all but one bed bug had contacted the treated panel and had been exposed to a lethal dose. Similar results were observed in the bifenthrin bioassays (Figure 3b). More than 65% of the bed bugs were in contact with the treated panel during the first three time intervals. With the onset of mortality at 50 min the percentage of bed bugs on the treated panel decreased. Bed bug mortality in the bifenthrin assays reached 100% at the end of the 2 h test period. Bed bugs took longer to aggregate onto the treated panels in the deltamethrin bioassays (Figure 3c). Still, more than 50% of the bed bugs were in contact with the treated panel at the 30 min time period. With the onset of mortality (50 min), the percentage of live bed bugs on the deltamethrin treated panels declined to 46.7%. After the 2 h test period, there was 100% mortality.

The susceptible strain bed bugs were willing to contact the non-pyrethroid products as well. Seventy three percent of the susceptible strain bed bugs were in contact with fabric panels treated with N.I.C. 325 dust at the first 10 min counting period. Over 50% of the bed bugs remained in contact with the dusted fabric for the entire 2 h test period. No mortality was recorded during the N.I.C. 325 test. Susceptible strain bed bugs were also willing to contact panels treated with chlorfenapyr. At the 30 min counting period, 50% of the bedbugs were in contact with the chlorfenapyr (Figure 3d) treated panel. Approximately 50% of the bed bugs remained in contact with the treated panel throughout the remainder of the test. There was also no mortality recorded in the chlorfenapyr bioassays during the 2h test period.

*Choice test.* The pairwise comparisons determined that the susceptible strain bed bug distribution between the treated and control panels within each of the bioassay arenas was not

significantly different for any of the pyrethroid formulations tested. These results indicated that susceptible strain bed bugs were not repelled by the pyrethroid treated panels, and that they contacted the treated and control panels equally (Dunnett's adjusted  $P > 0.61$ ). The overall comparison of bed bug response to panels (over the first 4 counting periods) in the choice tests indicated that there was no difference in the way that susceptible strain bed bugs responded to any of the pyrethroids tested (Figure 4a-e;  $df = 20$ ,  $F = 0.79$  and  $P = 0.57$ ).

There were no significant differences among the distribution of the field strain bed bugs on the treated and control panels for any of the pyrethroid formulations tested (Dunnett's adjusted  $P > 0.33$ ). These results indicated that field strain bed bugs also were not repelled by the pyrethroid treated panels, and were equally distributed between the treated panels and control panels. The overall comparison of field bed bug response to panels (over the first 6 counting periods) in the choice tests indicated that there was no difference in the response to any of the pyrethroids tested (Figure 5a-e;  $df = 20$ ,  $F = 0.81$  and  $P = 0.56$ ).

## **Discussion**

When comparing the bed bug response to the different pyrethroid products, the most striking discovery was that the field strain bed bugs were significantly less susceptible than the laboratory strain bed bugs. Cochran (1993) stated that if a susceptibility of field strain insects is ten times greater than that of a susceptible strain, the insects are classified as resistant and you can expect control failures. The  $LT_{50}$  value calculated for field strain bed bugs exposed to deltamethrin was significantly greater than that of the laboratory strain bed bugs, with a resistance ratio of over 450. The resistance ratio for field strain bed bugs exposed to bifenthrin was 99, indicating that the field strain was also highly resistant to bifenthrin. The cyfluthrin  $LT_{50}$

value for the field strain bed bugs was 17 times greater than the calculated  $LT_{50}$  value of the susceptible strain bed bugs, indicating that the field strain bed bugs were also resistant to cyfluthrin. The  $LT_{50}$  value for lambda-cyhalothrin in the field strain bed bugs was over 3 times greater and significantly different from that of the susceptible laboratory strain bed bugs. However, a three fold difference in susceptibility is not classified as resistance and we would not expect that this difference would result in control failures. These results indicate that field populations may be able survive relatively long periods of exposure to cyfluthrin, bifenthrin and deltamethrin, and possibly other pyrethroids currently being used for bed bug control.

Bed bugs have a history of resistance to multiple insecticide products (Barnes 1946, 1959, Rao and Halgeri 1956). Feroz (1974) documented bed bug resistance to malathion and fenchlorphos, but was unable to discover the specific detoxification enzymes responsible. Feroz (1974) measured the total esterase activity and acetylcholinesterase, but found no differences between a resistant strain and susceptible strain of bed bugs. Busvine (1959) tested DDT, methoxychlor, and analogues on Israeli strain bed bugs and found that bed bug “defense mechanisms” were similar to those found in *Anopheles gambiae* and *Musca domestica*, but Busvine (1959) did not specifically state what those specific mechanisms were. Busvine (1959), however, did conclude that there was no dehydrochlorination detoxification mechanism because DDT-resistant bed bugs were not overcome by the addition of DDT synergist.

Resistance to currently labeled insecticides is also a concern. Potter (2006b) found that adult bed bugs from four field locations in Kentucky and Ohio demonstrated > 1000 fold resistance to deltamethrin and lambda-cyhalothrin when compared to a susceptible strain. Using a discriminating dose assay on bed bug nymphs, Potter (2006b) also found > 100 fold resistance to deltamethrin in bed bugs from California, Florida, Kentucky, Ohio, and Virginia. The Potter

(2006b) results suggest that pyrethroid resistance in bed bugs may be widespread throughout the United States. These results also suggest that  $LT_{50}$  data for susceptible strain bed bugs may not be a good predictor for pyrethroid efficacy in the field.

Both the N.I.C. 325 dust and chlorfenapyr are new bed bug products with no history of use for bed bug control. Therefore, no resistance to either product was expected or demonstrated in preliminary laboratory tests. However, the  $LT_{50}$  values calculated for these novel products were surprisingly high compared to the pyrethroid products. While pyrethroid  $LT_{50}$  values were calculated in hours, N.I.C. 325 took 9 d 10 h to kill the laboratory strain bed bugs. Marketing materials for the N.I.C. 325 dust state that the product is a desiccant that abrades the bed bug cuticle. However our data indicated that N.I.C. 325 take  $> 7$  days to kill bed bugs even when the insects are covered in dust. The fact that bed bugs are not readily susceptible to a non-insecticidal desiccant dust is not surprising when you consider that bed bugs can live for extended periods of time (18 months) without feeding (Bacot 1914). Because the blood meal is the only source of moisture and bed bugs can survive long periods of starvation suggests that the bed bug cuticle may be specialized to prevent moisture loss. Therefore, this cuticular specialization may reduce the susceptibility of desiccant dusts like N.I.C. 325.

The  $LT_{50}$  values calculated for chlorfenapyr were exceptionally high (10 d 9 h) when compared with the pyrethroids. In fact, chlorfenapyr allowed the bed bugs confined on the treated panels enough time to mate and lay eggs. The mean number of eggs laid in the chlorfenapyr bioassays (75.2 eggs in 120 h) was not significantly different from those laid in the control bioassays (45.3 eggs in 120 h). All of the eggs hatched in both groups. Many of the nymphs exposed to chlorfenapyr survived after removal from the treated panels. Chlorfenapyr's lack of efficacy is most likely due to its mode of action. Chlorfenapyr is primarily a stomach

toxicant which uncouples oxidative phosphorylation by disrupting the proton gradient formation (Ware and Whitacre 2004). Ultimately, the mode of action is that chlorfenapyr prevents the formation of ATP. Because chlorfenapyr is a stomach toxicant, ingestion is the key exposure route. Because bed bugs do not groom like cockroaches, chlorfenapyr residues on the treated panel have no means of being ingested. Chlorfenapyr did kill bed bugs eventually, but because it is so slow acting it might not be acceptable to pest control operators and their clients as a stand-alone bed bug treatment.

None of the insecticides tested, including the pyrethroids, were repellent to the susceptible or field strain bed bugs. Both strains of bed bugs readily contacted surfaces treated with pyrethroids. These results were interesting because many studies have shown that pyrethroid-type chemistry is repellant to other crawling household pests such as cockroaches (Ebeling et al. 1966), termites (Smith 1979), and ants (Knight and Rust 1990). However, these results were consistent with Usinger's (1966) statement that "all common bed bug repellents were tested and none prevented bed bugs from crossing a treated ring around a simulated bed post" (Berryman, cited by Usinger (1966)). Usinger (1966) reported that 15% deet did give complete protection for up to 5 hours and fair protection for 7 hours. Therefore, pyrethroids can be used without concern about scattering bed bug aggregations or causing bed bugs to avoid treated surfaces.

**Table 3.1.** Time to mortality of susceptible strain and field adult bed bug confined on insecticide treated hardboard panels.

Treatment	N	LT <sub>50</sub> % Hour*	95% CIs	Slope $\pm$ SE	$\chi^2$ (df)
<i>Susceptible strain</i>					
$\lambda$ -Cyhalothrin (0.03%)	50	0.34a	0.32 - 0.36	12.56 $\pm$ 2.4	0.01 (18)
Cyfluthrin (0.06%)	50	0.73b	0.70 - 0.76	16.9 $\pm$ 2.1	26.15 (33)
Bifenthrin (0.02%)	50	0.89c	0.82 - 0.96	9.24 $\pm$ 0.9	8.45 (38)
Deltamethrin (0.06%)	50	1.01d	1.00 - 1.15	7.35 $\pm$ 0.6	9.00 (48)
Permethrin (0.05%)	50	1.46e	1.37 - 1.56	10.17 $\pm$ 0.9	15.43 (53)
N.I.C 325	50	227.03h	206.4 - 252.6	2.41 $\pm$ 0.2	41.40 (93)
Chlorfenapyr (0.5%)	50	243.69h	221.6 - 264.7	5.21 $\pm$ 0.4	33.31 (12)
Control **					
<i>Field strain</i>					
$\lambda$ -Cyhalothrin (0.03%)	50	1.18d	1.11 - 1.24	4.78 $\pm$ 0.26	102.3 (108)
Cyfluthrin (0.06%)	50	12.17f	10.25 - 14.59	1.34 $\pm$ 0.07	86.3 (103)
Bifenthrin (0.02%)	50	88.18g	77.32 - 101.2	2.45 $\pm$ 0.25	80.9 (108)
Deltamethrin (0.06%)	50	457.94i	328.27 - 517.7	2.52 $\pm$ 0.15	436.1 (153)
Control **					

\*Means followed by different letters are significantly different (PoloPlus 2004).

\*\* No control mortality was recorded

**Figure Legend.**

**Figure 3.1.** Diagram of repellency bioassay arena- Non-choice test. page 50

**Figure 3.2.** Diagram of repellency bioassay arena- Choice test. page 50

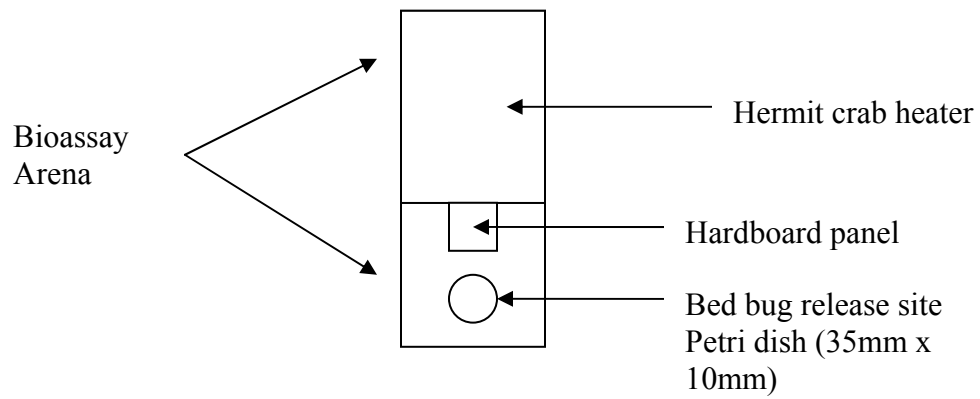
**Figure 3.3a-3.3e.** Mean percent ( $\pm$  SE) of laboratory strain bed bugs aggregating on treated panels in non-choice repellency assays. page 51

**Figure 3.4a-3.4e.** Mean percent ( $\pm$  SE) bed bug response to treated and untreated panels in choice repellency assays. page 56

**Figure 3.5a-3.5e.** Mean percent ( $\pm$  SE) bed bug response to treated and untreated panels in pyrethroid bioassays. page 61



**Figure 3.1 Diagram of repellency bioassay arena- Non-choice test.**



**Figure 3.2 Diagram of repellency bioassay arena- Choice test.**

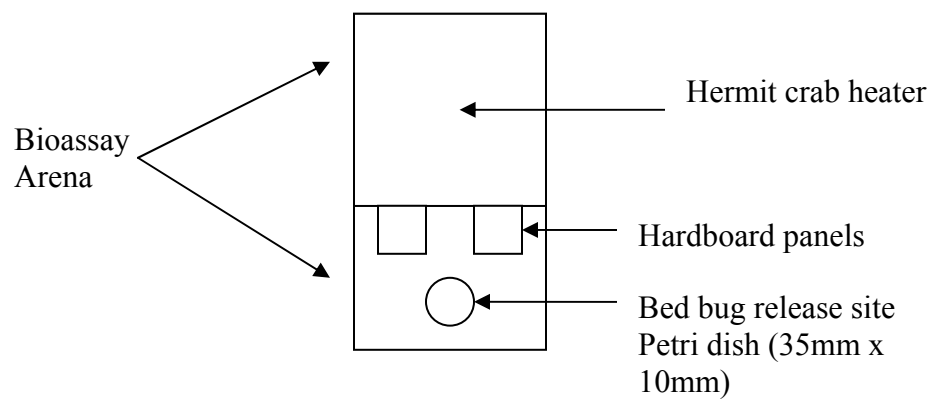


Figure 3.3a Mean percent (+ SE) of laboratory strain bed bugs aggregating on treated panels in non-choice repellency assays.

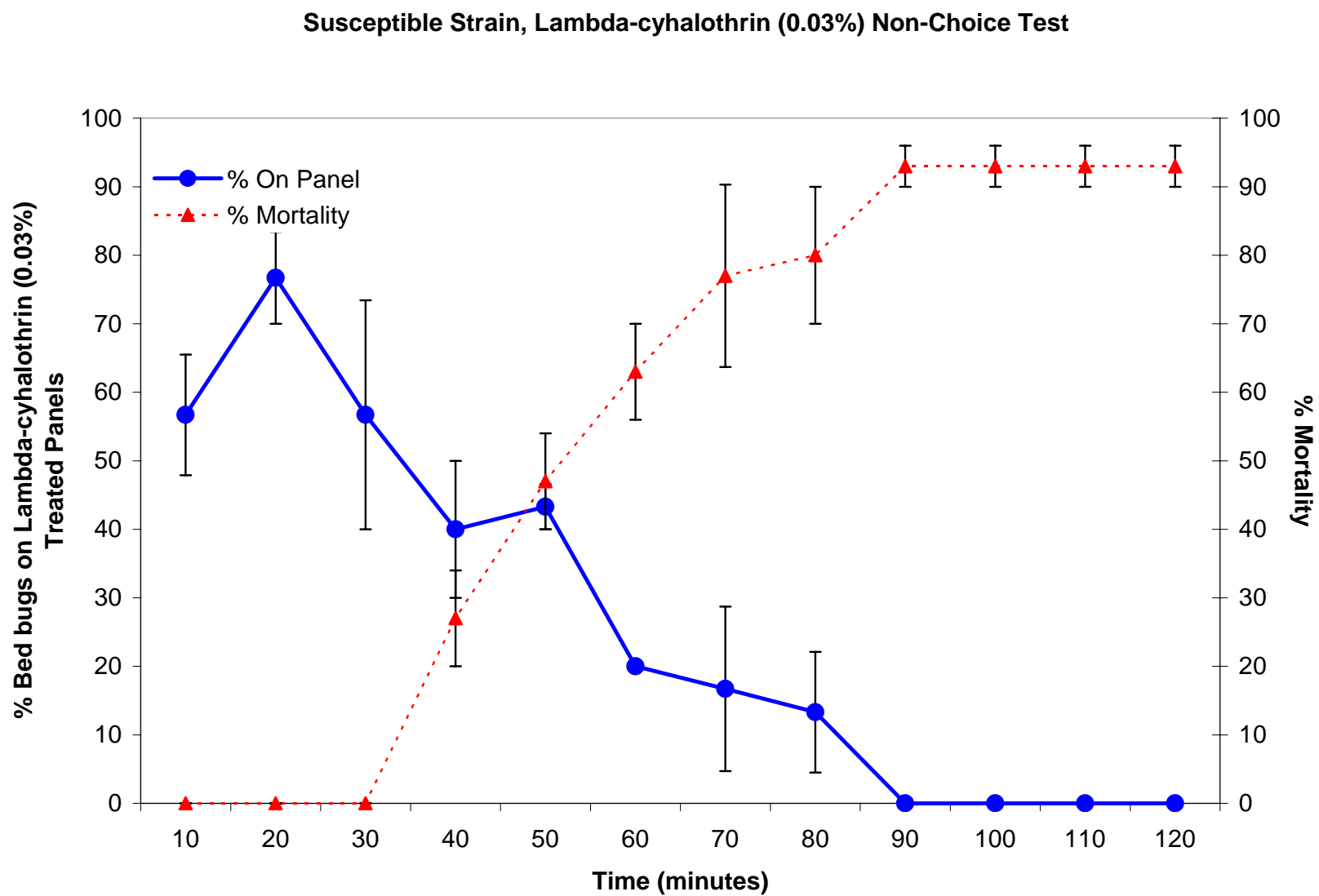


Figure 3.3b Mean percent (+ SE) of laboratory strain bed bugs aggregating on treated panels in non-choice repellency assays.

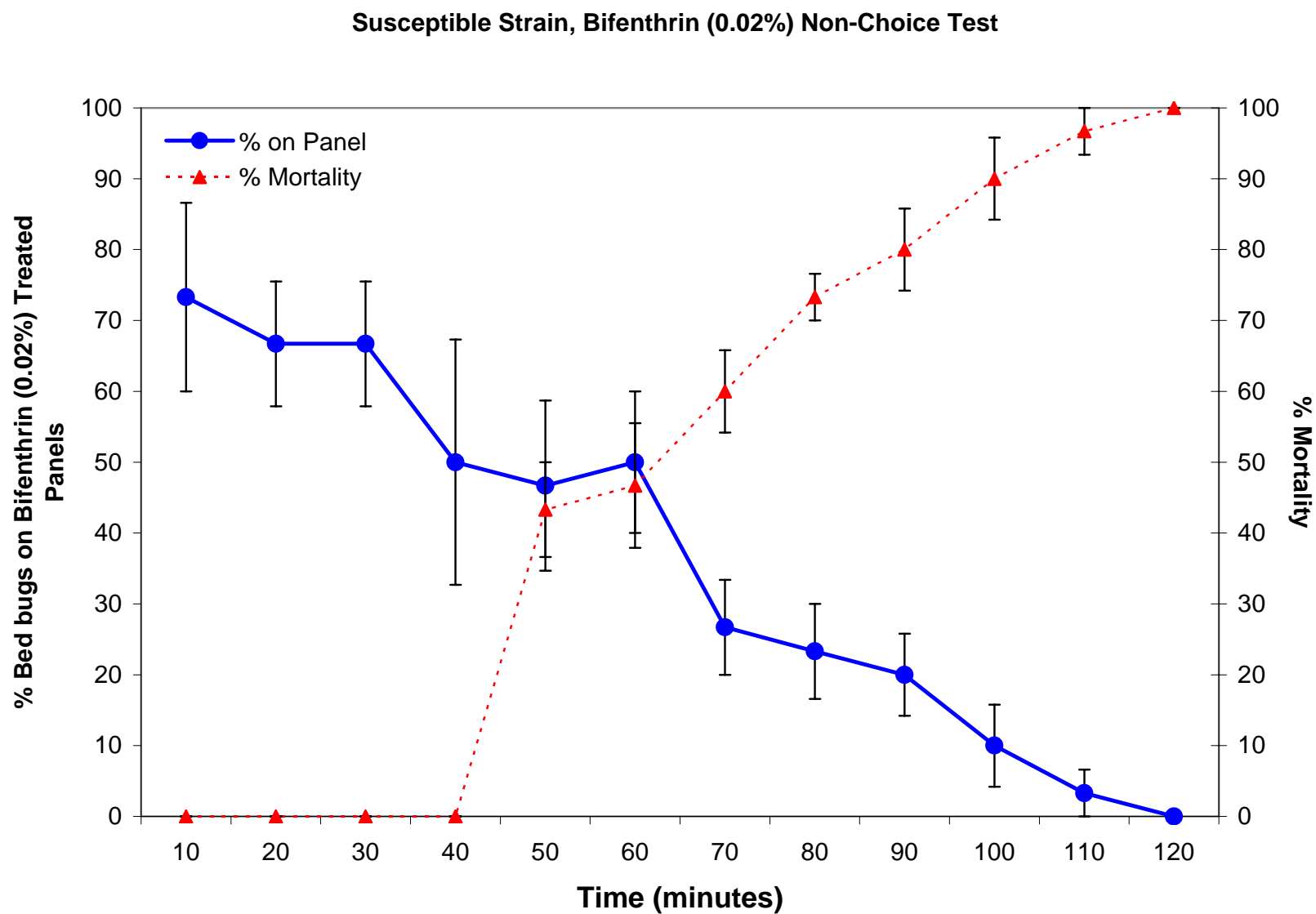


Figure 3.3c Mean percent (+ SE) of laboratory strain bed bugs aggregating on treated panels in non-choice repellency assays.

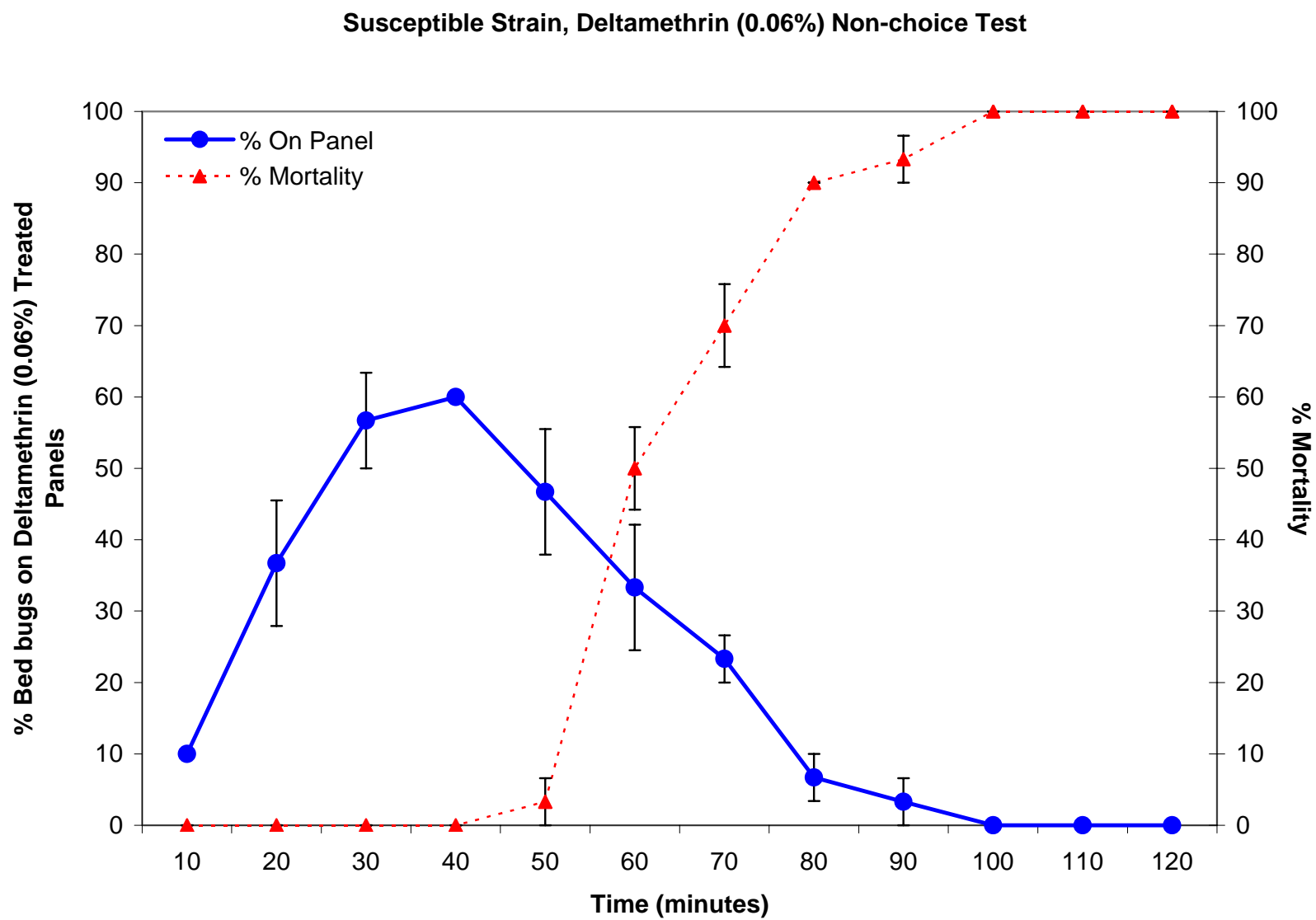


Figure 3.3d Mean percent (+ SE) of laboratory strain bed bugs aggregating on treated panels in non-choice repellency assays.

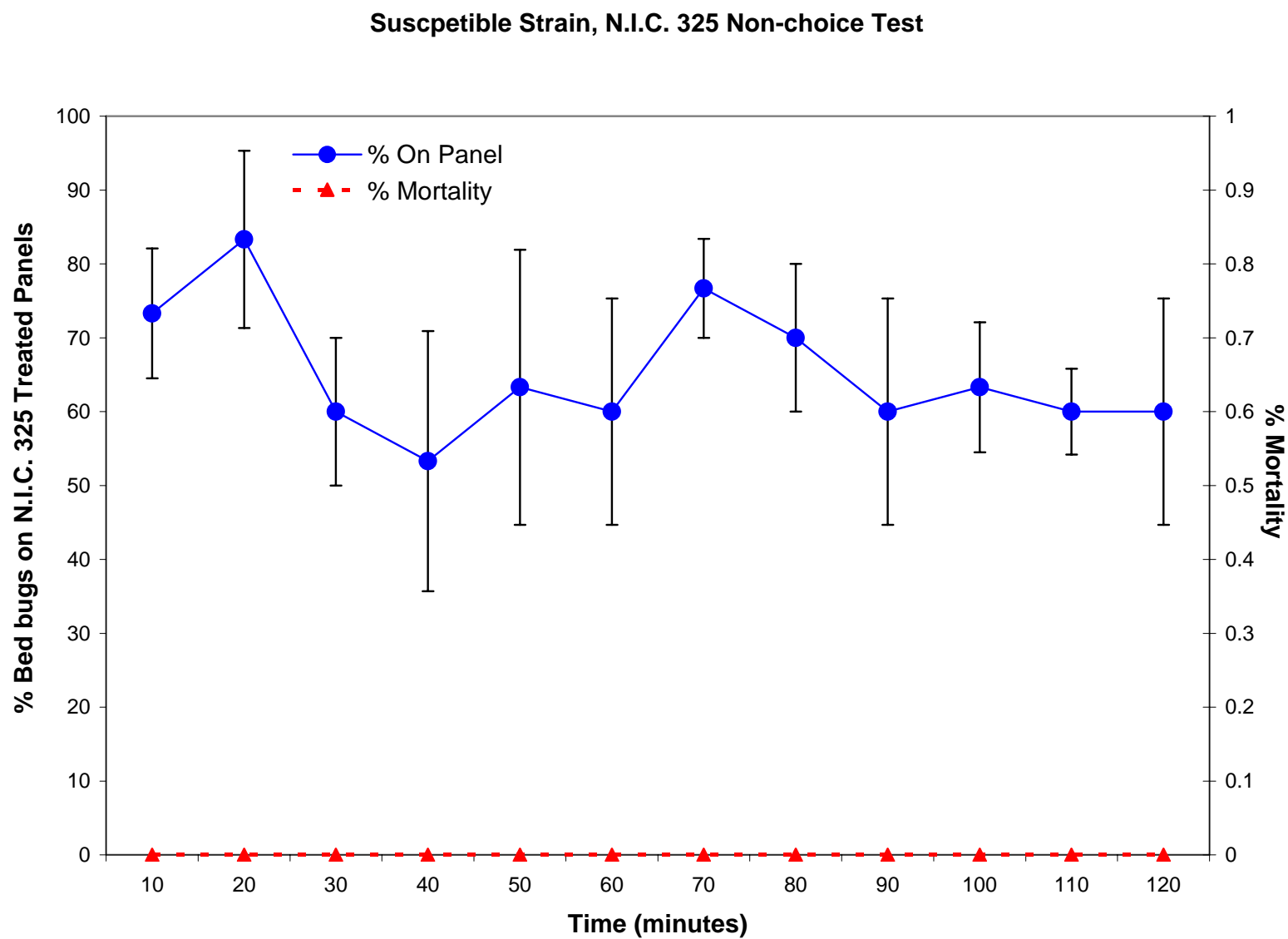


Figure 3.3e Mean percent (+ SE) of laboratory strain bed bugs aggregating on treated panels in non-choice repellency assays.

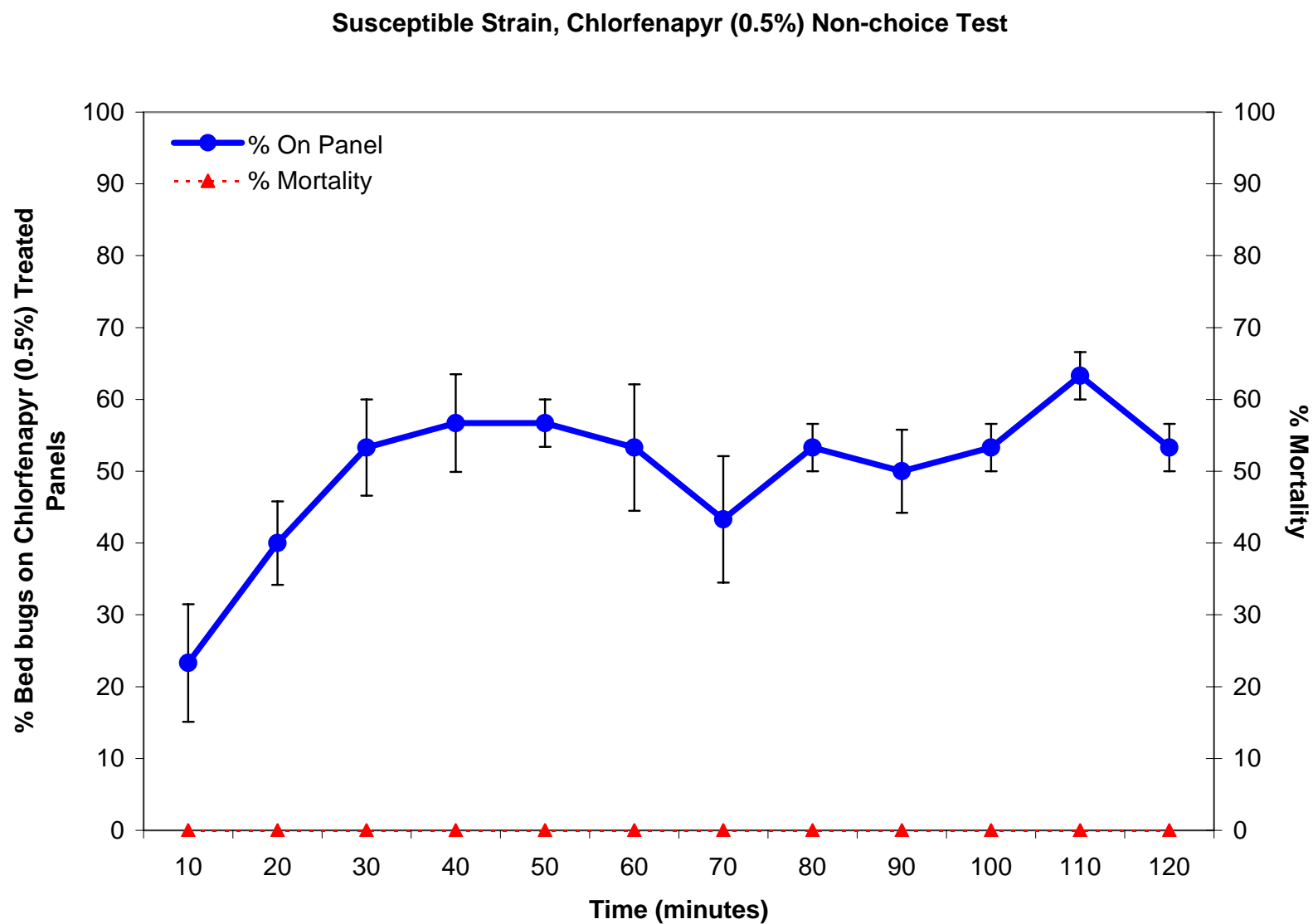


Figure 3.4a Mean percent (+ SE) bed bug response to treated and untreated panels in choice repellency assays.

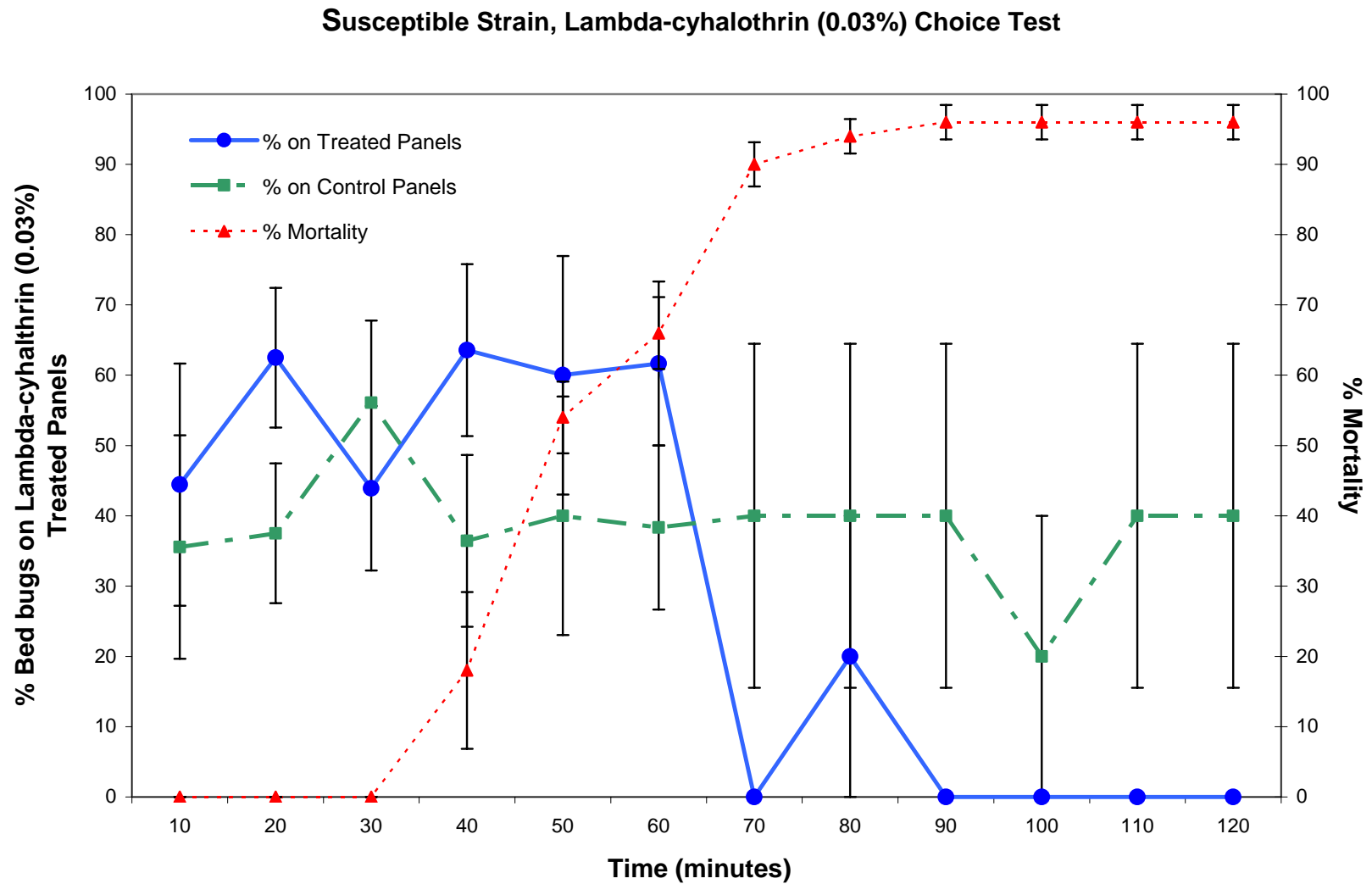


Figure 3.4b Mean percent (+ SE) bed bug response to treated and untreated panels in choice repellency assays.

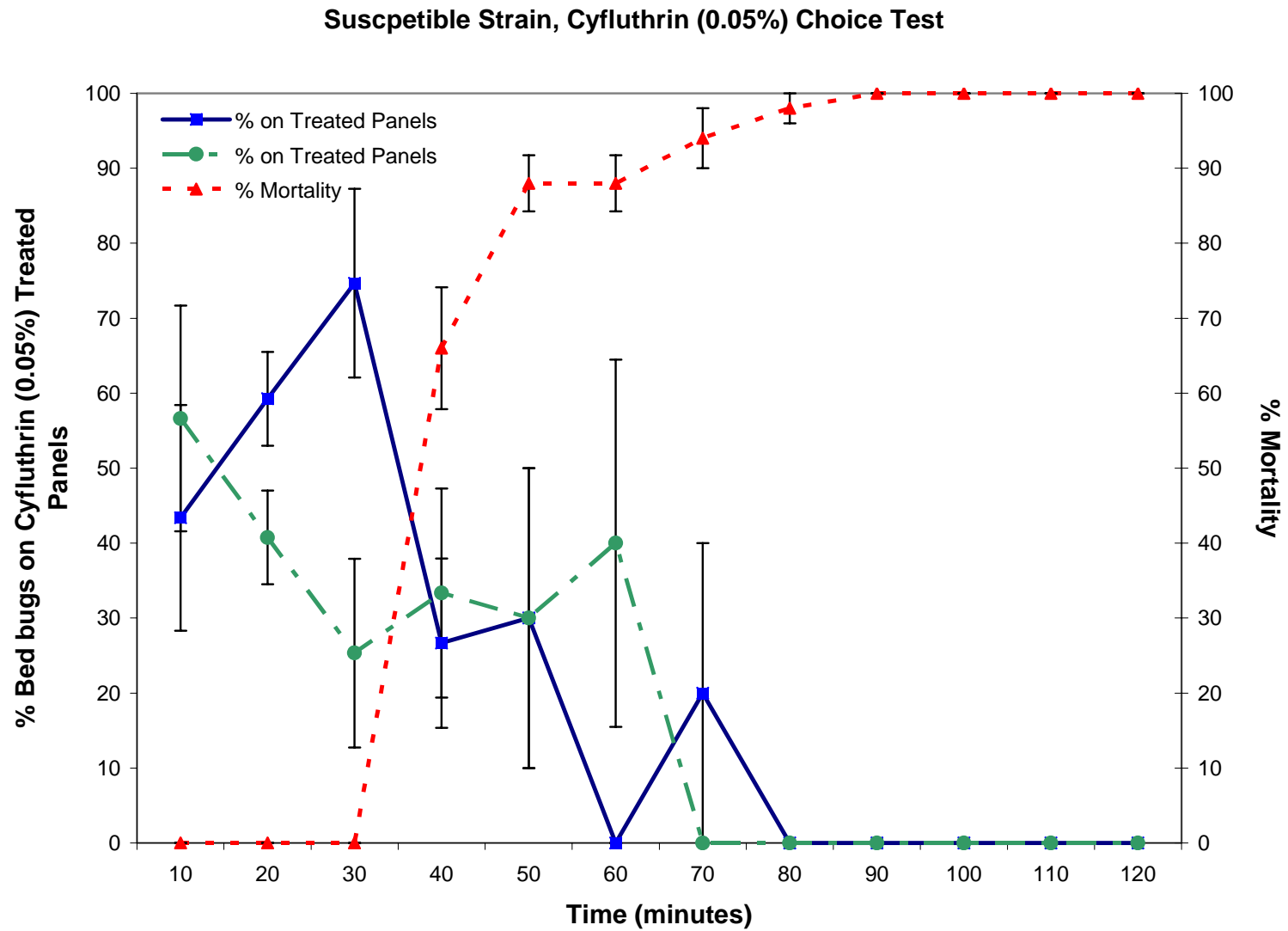




Figure 3.4c Mean percent (+ SE) bed bug response to treated and untreated panels in choice repellency assays.

**Susceptible Strain, Bifenthrin (0.02%) Choice Test**

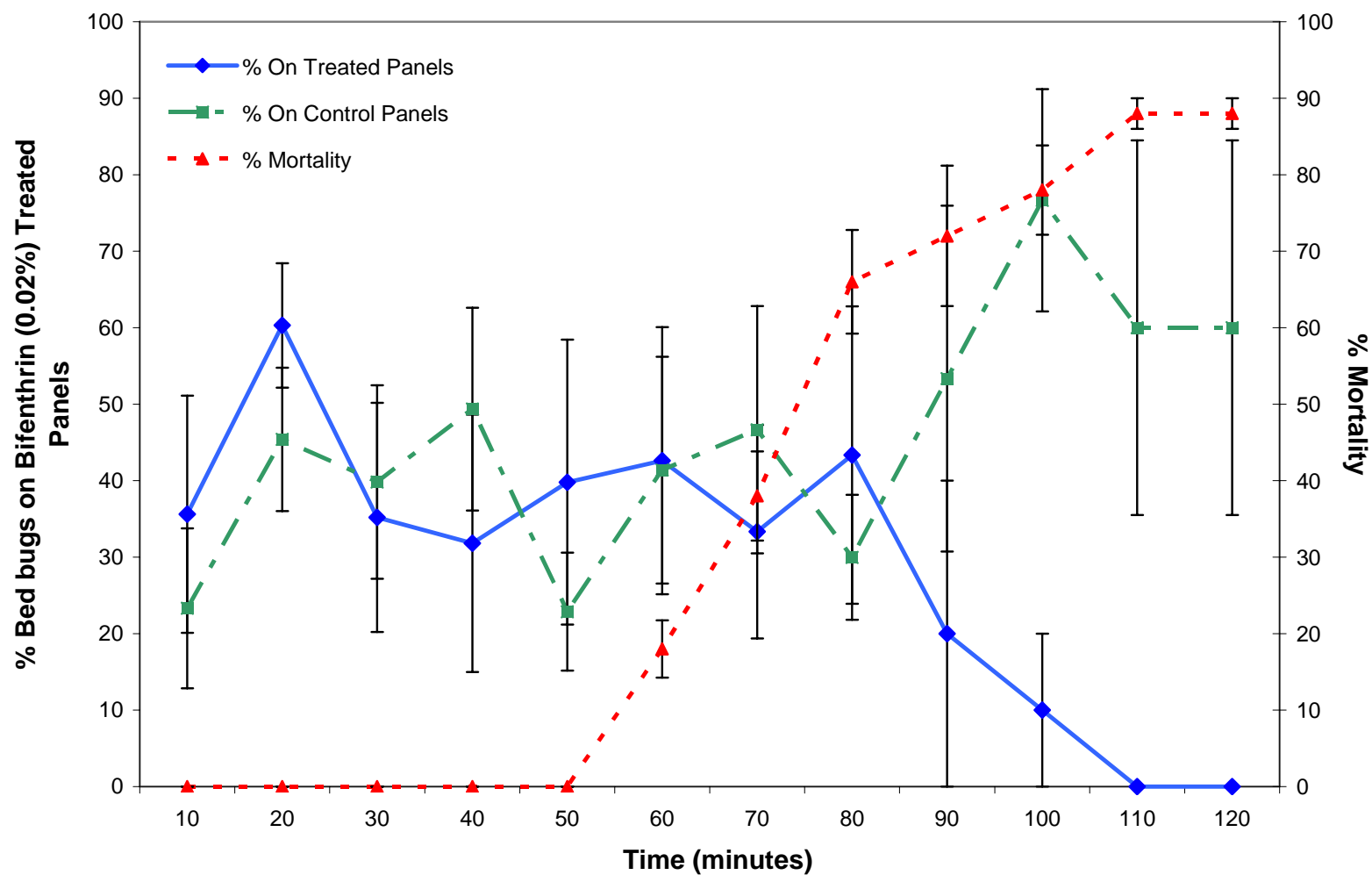


Figure 3.4d Mean percent (+ SE) bed bug response to treated and untreated panels in choice repellency assays.

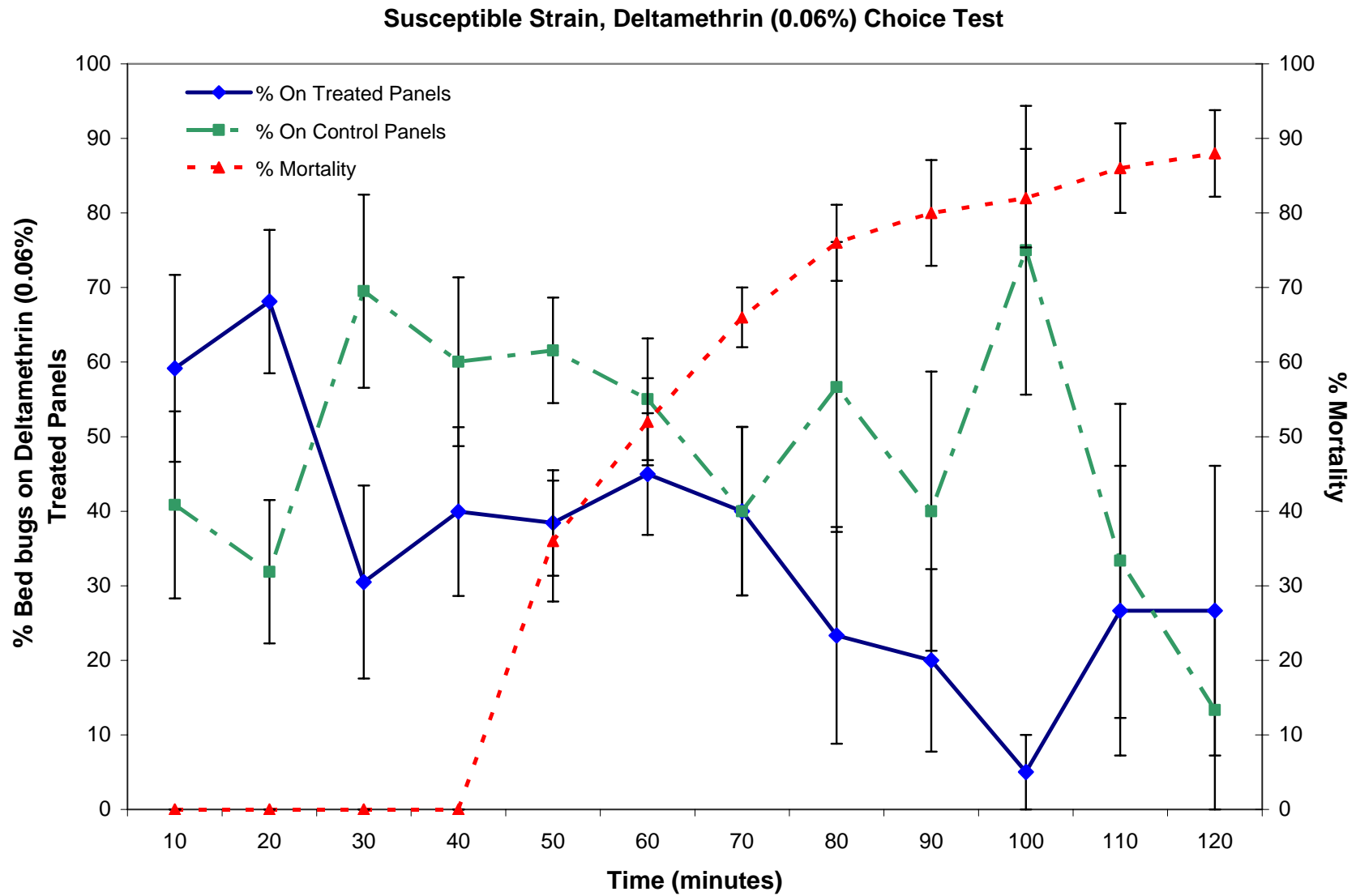
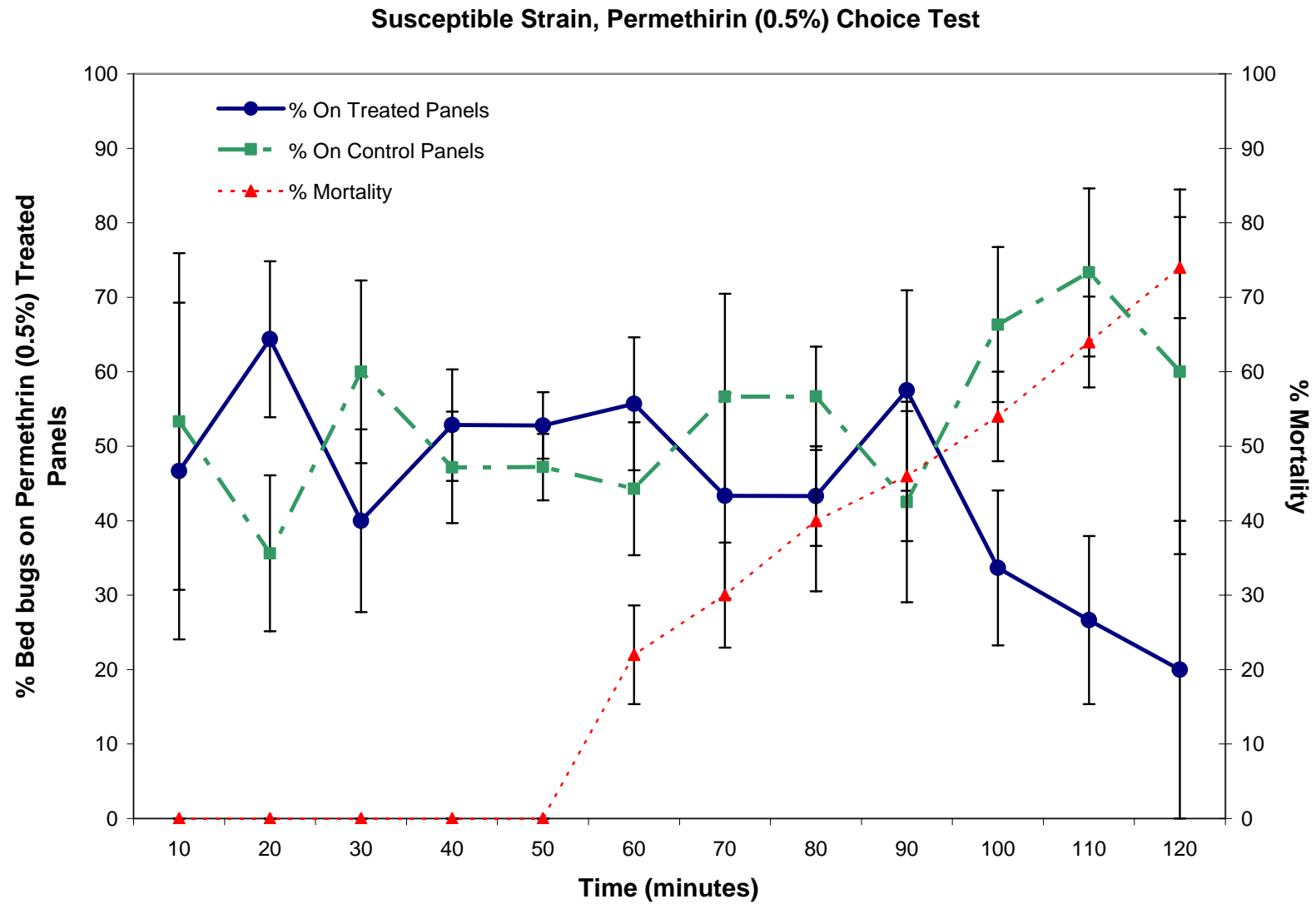


Figure 3.4e Mean percent (+ SE) bed bug response to treated and untreated panels in choice repellency assays.



**Figure 3.5a** Mean percent (+ SE) bed bug response to treated and untreated panels in pyrethroid bioassays.

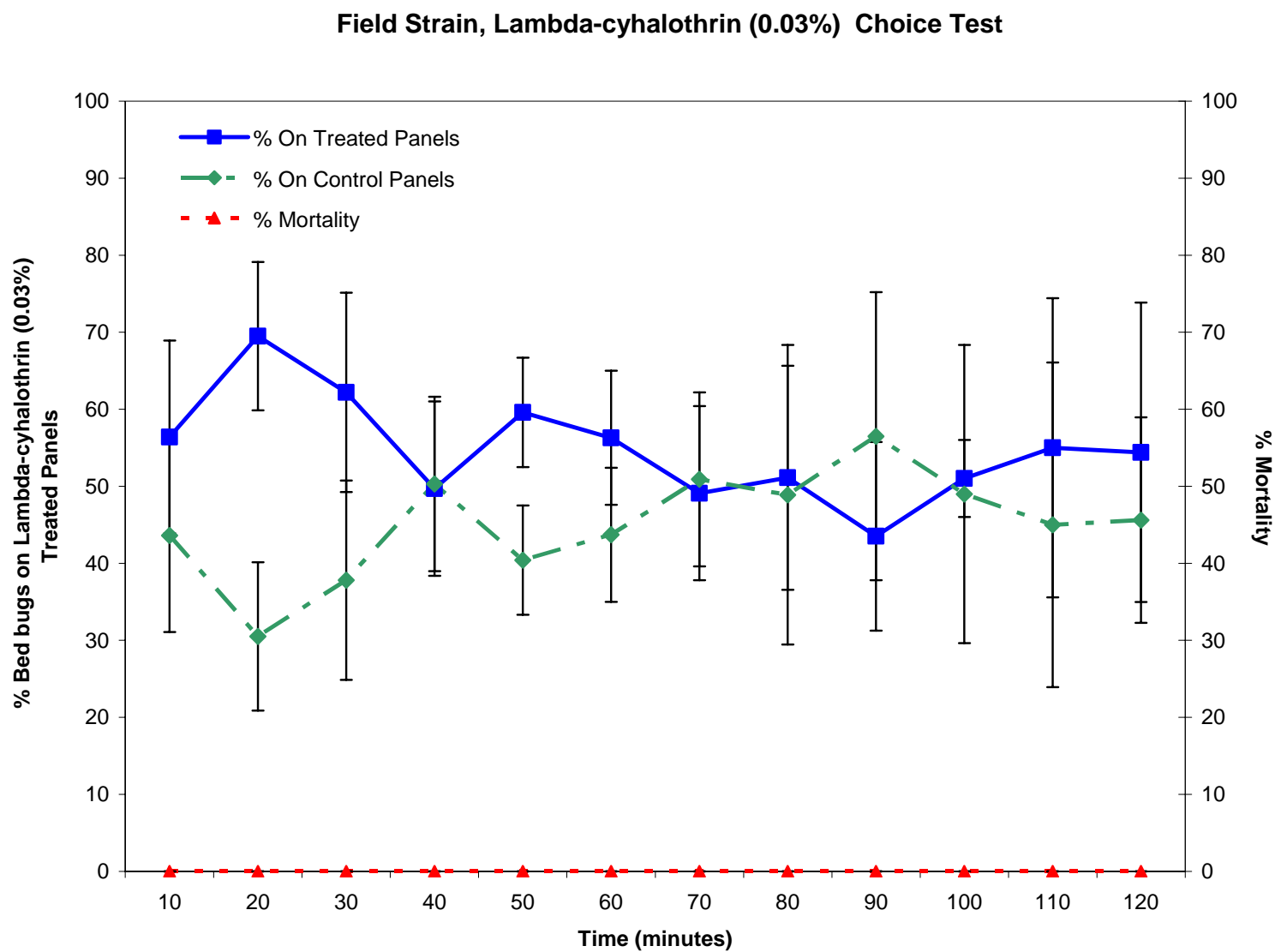


Figure 3.5b Mean percent (+ SE) bed bug response to treated and untreated panels in pyrethroid bioassays.

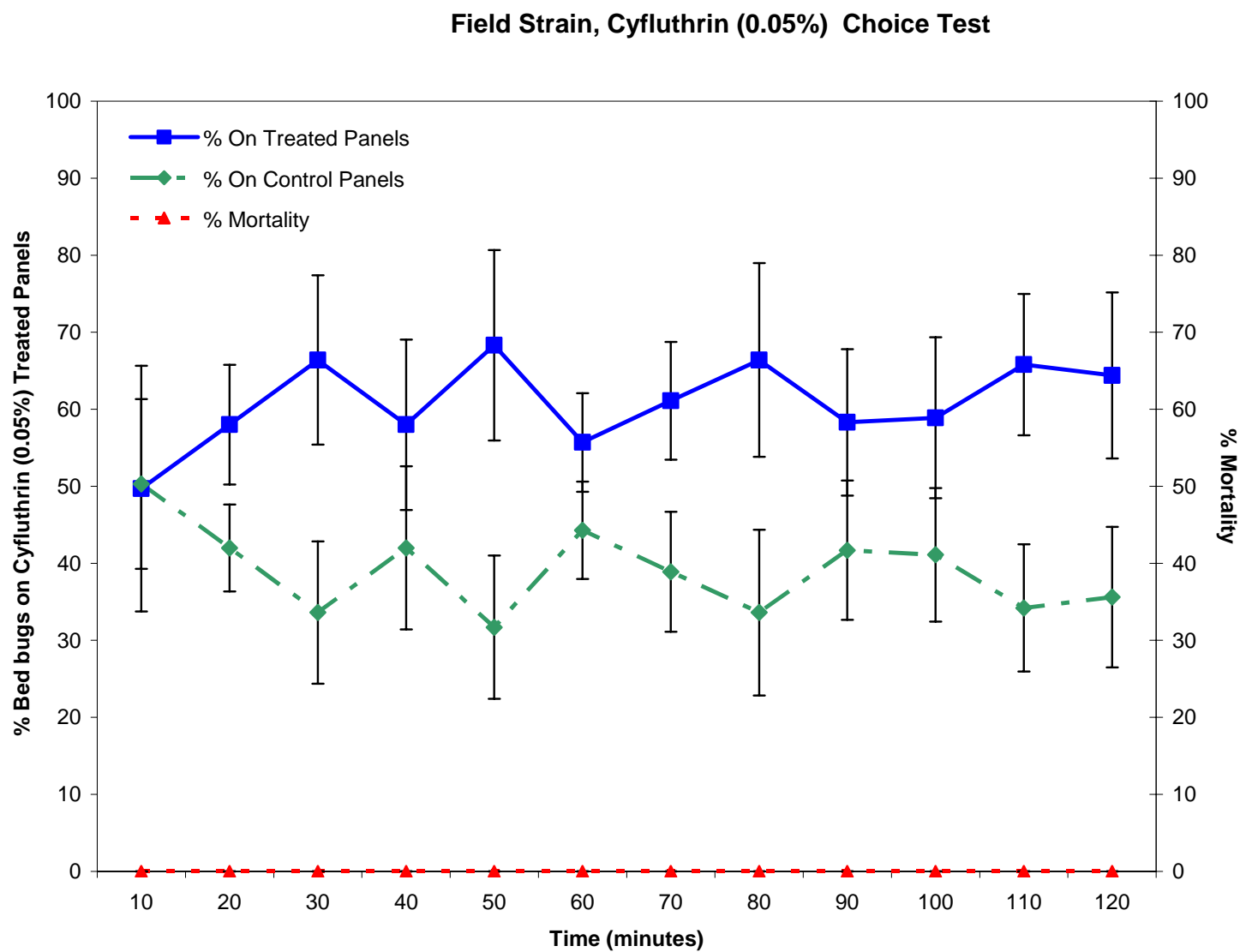


Figure 3.5c Mean percent (+ SE) bed bug response to treated and untreated panels in pyrethroid bioassays.

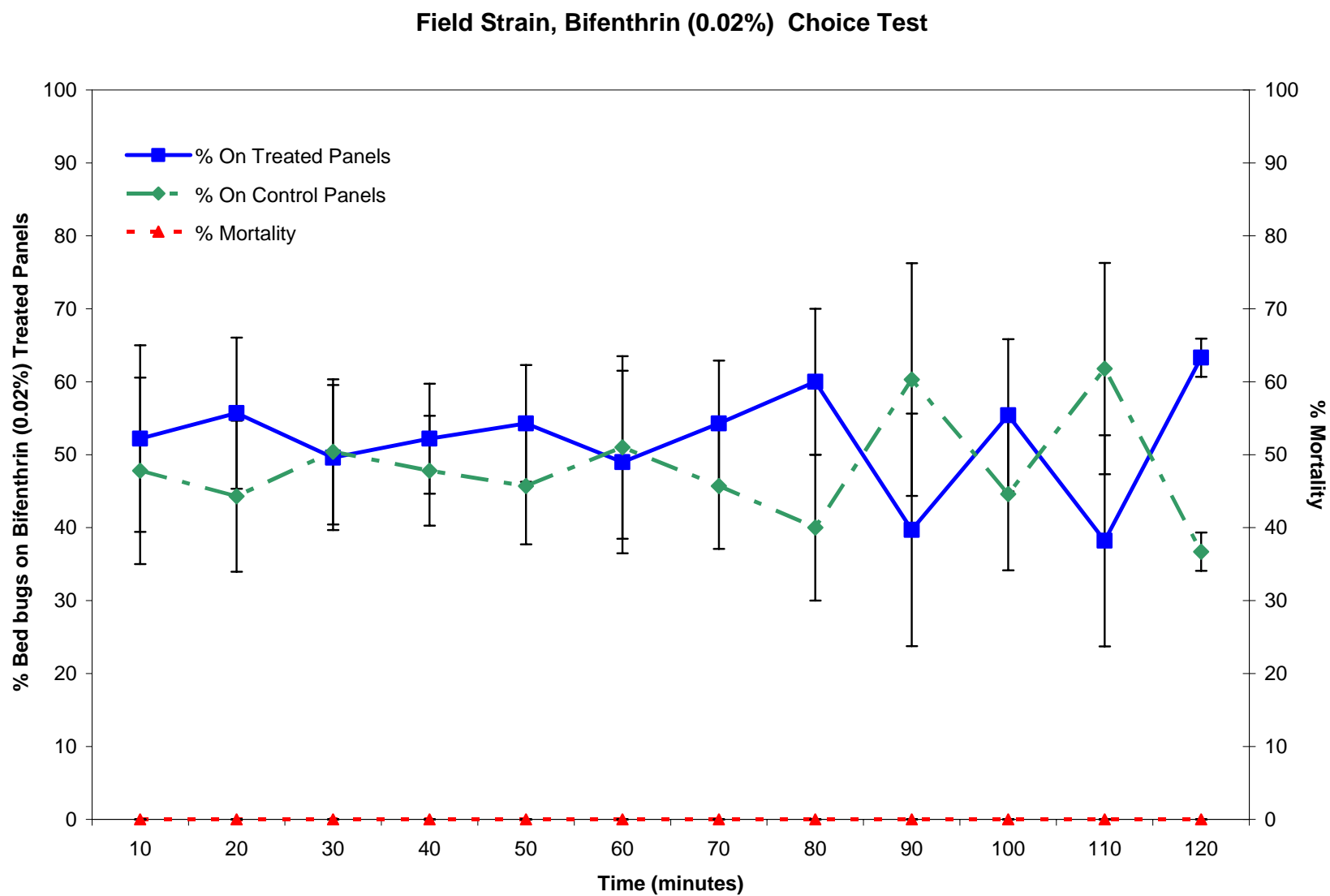
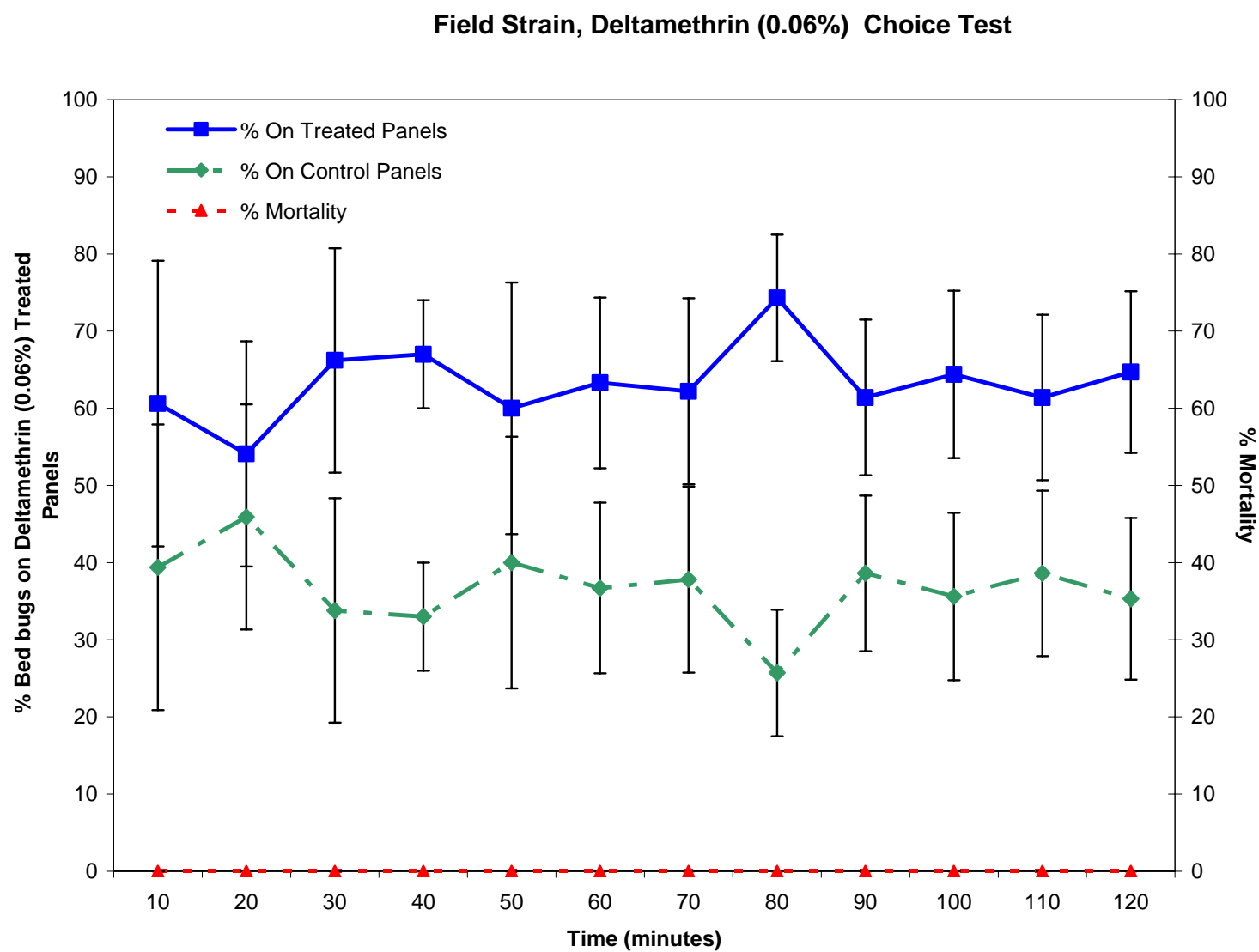
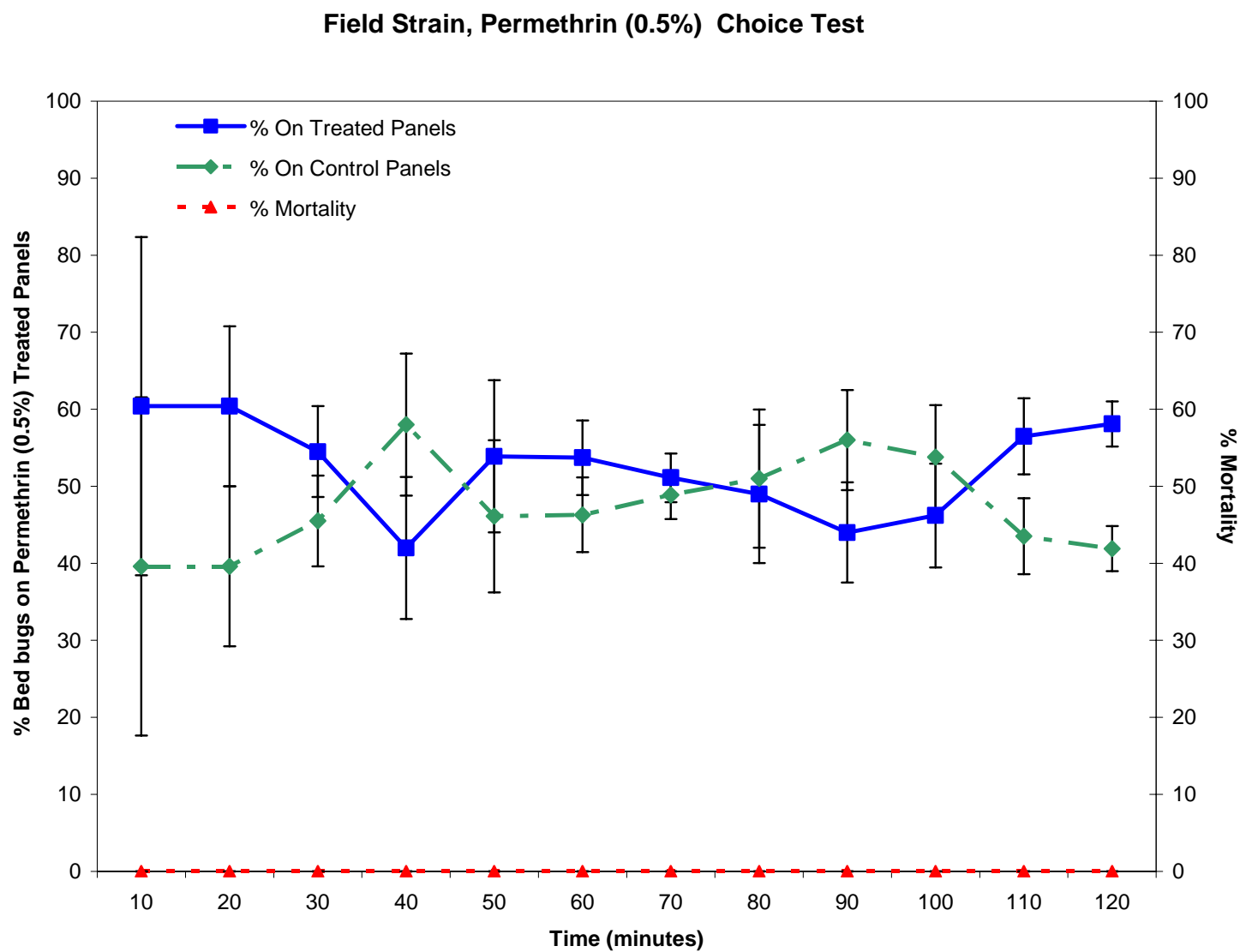


Figure 3.5d Mean percent (+ SE) bed bug response to treated and untreated panels in pyrethroid bioassays.



**Figure 3.5e** Mean percent (+ SE) bed bug response to treated and untreated panels in pyrethroid bioassays.





## **Chapter Four**

### **The Evaluation of Two Treatment Combinations for the Control of Bed Bugs in Low Income Apartments**

#### **Introduction**

The common bed bug, *Cimex lectularius*, is becoming a major household pest across North America, with the number of reported infestations in single family homes, hotel rooms, and multi-family units increasing every year since 1990 (Cooper and Harlan 2006). Bed bugs can be introduced into new locations in several ways, e.g. with the introduction of infested furniture, in the luggage of travelers, or by purchasing mattresses that have been “refurbished” (Harlan 2006a, Kells 2006a, Usinger 1966, WHO 1997). In addition, bed bugs have been documented moving from one apartment unit into an uninfested neighboring apartment in multi-unit housing (Usinger 1966).

Bed bugs are highly cryptic and can hide almost anywhere (Boase 2004), including the cracks and crevices of bedding, furniture, walls, floors, behind picture frames, under wallpaper, behind light switches, in door and window frames, and behind baseboards (Hwang 2005, Gunn 1933, Usinger 1966). Bed bugs prefer areas of darkness (Hase 1917) with little air flow (Kemper 1936) and minimal disturbance (Cooper 2006). Bed bug harborages are not evenly distributed throughout the host’s dwelling (Usinger 1966), but are typically located near the bed or in close proximity of their host. However, in heavily infested dwellings, bed bug aggregations can be found throughout the home.

Currently, there are no commercial monitoring devices available for bed bug detection (Kells 2006a). Initial detection of a bed bug infestation usually starts with the host complaining of bites. Additional evidence of an infestation might include blood spotting (Doggett et al 2004) and excrement deposits on the mattress or wall (WHO 1997), exuvia in harborage locations

(Gunn 1933), or the presence of live or dead bed bugs. Typically, evidence of a bed bug infestation (other than bites) does not become visible until the infestation has been present for some time. Unless a pest management professional has had experience in dealing with bed bug infestations, it is often will be difficult for them to locate bed bug harborages and control the infestation (Doggett et al 2004).

Known as “the bug that nobody knows,” bed bugs are typically viewed as an embarrassment (Usinger 1966). Due to the social stigma associated with bed bugs, tolerance is essentially zero. As a result, the hospitality and housing industry often refuses to discuss or admit to a bed bug infestation for fear of bad publicity or litigation (Potter 2006). Homeowners are also embarrassed by bed bugs and do not want other people to know of their infestation. As a result, homeowners will often try to eliminate the infestation by themselves (Potter 2006). However, out of ignorance, the homeowner’s efforts often fail to correct the problem, thus allowing the infestation to perpetuate. Bed bug elimination is a daunting task, and even pest management professionals have a difficult time eliminating bed bugs from a location (Potter 2006).

Humans have been struggling with bed bug control for centuries (Usinger 1966), with little relief until the introduction of dichloro-diphenyl-trichloroethane (DDT) in 1939 (Ebeling 1975). Although DDT was initially very successful for bed bug control, within eight years bed bugs had developed resistance (Johnson and Hill 1948). However, the liberal use of malathion and DDT practically eliminated bed bugs in the United States by the 1960s (Usinger 1966). Eventually however, bed bugs developed resistance to a host of chemicals used for their control. These chemicals included methoxychlor, dieldrin, aldrin (Busvine 1980; Gaaboub 1971), carbaryl, endrin, gamma BHC (Radwan et al. 1972), sodium fluoride (Feroz 1974), malathion

and other organophosphates, benzene hexachloride and other chlorinated cyclodienes (Usinger 1966). Many of these older chemistries have lost their registration with the U. S. Environmental Protection Agency and are no longer available for bed bug treatment.

Because bed bugs have not been a problem in the U.S. for over 40 years, few of the newer insecticide products are labeled for bed bug control. The majority of insecticide products that are labeled for the treatment of bed bugs are either natural pyrethrins or synthetic pyrethroids. However, there are several non-pyrethroid products that are currently being used alone or in combination with other products for bed bug control in the field. Although laboratory assays have been conducted to evaluate the efficacy of pyrethroids and some of the novel products currently available for bed bug control (Moore and Miller 2006), these insecticides have not been evaluated for their field efficacy.

The purpose of the study presented here was to evaluate the field efficacy of two different product combinations intended to control bed bugs. Each of the treatment combinations included the use of multiple products currently labeled for bed bug control.

### **Materials and Methods**

**Field Site.** Evaluations of bed bug treatments were conducted from January to March 2006 in Buckingham Village, a low income housing facility located in Arlington, VA. The facility consisted of two-story brick buildings, containing eight to sixteen apartment units on a slab foundation. The housing facility was built between 1937 and 1953 and has been under numerous pest control contracts. The current pest control contract requires that each apartment unit be treated on a quarterly basis for general household pests. Treatment consists of a combined application of Demand CS (lambda-cyhalothrin 0.03%), Gentrol (hydroprene 1oz/1500 sq ft), and Maxforce FC (Fipronil, 0.01%). When the facility had a bed bug complaint,

the pest management company applied a supplementary treatment using a combination of Demand CS (lambda-cyhalothrin 0.03%), Suspend SC (deltamethrin 0.06%), and Gentrol (hydroprene 1oz/1500 sq ft.

The residents of Buckingham Village were primarily Hispanic day-laborers who were normally working during the treatment hours. The typical absence of residents coupled with the language barrier meant that little advice could be provided on sanitation or bed bug prevention to the tenants.

The living situation of the residents in this facility was somewhat unique. Typically, there was a single resident holding the lease for the apartment, while the other tenants rented from the lease holder at a daily or weekly rate. This arrangement allowed tenants to move themselves and their belongings from one apartment to another every few days. The overcrowded conditions in the housing facility and frequent movement of the residents contributed to the bed bug problems.

Tenants in bed bug infested apartments frequently applied their own bed bug control measures. For example, boric acid was applied along the baseboards, inside bed frames, and other furniture. Masking or duct tape was also used along the baseboards, around wall heaters, electrical outlets, and switch plates to contain bed bugs inside the walls. Caulk was also used to seal bed bug harborages. Bleach water and consumer pyrethroid products were often applied on furniture and bedding to kill bed bugs.

**Apartment Selection.** During the initial inspection of the housing facility (January 2006), it was observed that the intensity of bed bug infestations varied from one apartment to the next. However, all apartment units had signs of bed bug infestation. To quantify the pretreatment bed bug infestation level, individual apartments were visually inspected. Kitchens

and bathrooms were typically dominated by German cockroaches and no bed bugs were found in these rooms during the initial inspection, therefore these rooms were neither inspected nor treated for the duration of the test. Areas used for sleeping rooms (i.e. bed rooms, living room, and dining room) in the apartment were inspected for bed bugs. Within each “sleeping room”, the number of bed bugs on the walls, baseboards, ceilings, bedding, and other furniture was recorded. Apartments in which > 10 live bed bugs were found were selected for use in this study. Fifteen apartments were selected to participate in the test and were randomly designated into one of the treatment groups or into the control group.

**Treatment Regimens.** Groups of five apartments were assigned to receive one of two bed bug treatment combinations. One treatment combination consisted of products which have been long established for bed bug control and have bed bugs on the label. This treatment was designated the “traditional treatment.” The second treatment combination consisted of newer products that have not been empirically tested for bed bug control, but have bed bugs on the label or a site label for crawling insect pests. This treatment combination was designated as the “novel treatment.”

*Traditional treatment.* The products selected for use in the traditional treatment were Tempo SC Ultra (0.05% beta-cyfluthrin, Bayer CropScience LP, Montvale, NJ.), Suspend SC (0.06% deltamethrin, Bayer CropScience LP, Montvale, NJ), and Gentrol Aerosol (0.36% hydroprene, Wellmark International Schaumburg, IL). Tempo SC Ultra was formulated in a B&G sprayer (1-gal Prime Line 2000, B&G Equipment Co. Jackson, GA) and applied to baseboards, ceiling/wall junctions, and cracks and crevices where bed bugs were harboring. Suspend SC was also formulated in a B&G sprayer and applied to mattresses and boxsprings. Gentrol Aerosol was applied around the baseboards, cracks and crevices, and as a border around

bed frames or boxsprings. After the initial treatment, each apartment was treated at two week intervals (maintenance treatments) with all of the products for the duration of the test.

*Novel treatment.* The products selected for use in the novel treatment were Phantom (aqueous solution of 0.5% chlorfenapyr, BASF Corporation Research Triangle Park, NC), Steri-Fab (60.39% Isopropyl alcohol, Noble Pines Products Co. Yonkers, NY), N.I.C. 325 (dust formulation of 99.5% limestone, AMC-Texas L.L.C. Fort Collins, CO), and Gentrol Aerosol (0.36% hydroprene, Wellmark International Schaumburg, IL). Phantom was formulated in a B&G sprayer (1-gal Prime Line 2000, B&G Equipment Co., Jackson, GA) and applied to baseboards, ceiling/wall junctions, and cracks and crevices where bed bugs were harboring. Steri-Fab was used only once during the initial treatment and was applied directly on bed bugs harboring along the tufts and seams of the mattress and boxsprings. N.I.C. 325 was used in subsequent mattress treatments and was applied on the seams and tufts on the mattress and boxsprings and inside the boxsprings. Gentrol Aerosol was also used in the novel treatment and applied as described in the traditional treatment. After the initial application, Phantom, Gentrol, and N.I.C. 325 were applied as maintenance treatments every two weeks for the duration of the test.

*Treatment and monitoring schedule.* Treatments were applied by trained, certified pest management personnel (PMPs) from Innovative Pest Management Co. and by David Moore of Virginia Tech, a certified technician. Both traditional and novel treatments were applied every two weeks for eight weeks. The first treatment application during the initial visit was intended to kill as many bed bugs as possible. Both the initial treatment and subsequent maintenance treatments allowed the PMPs to use as much product as they deemed necessary to control bed bug populations. Apartments were treated “as is.” No modifications were made to individual

apartments for treatments and the housing residents were not required to move objects away from the walls or to clean their units at any time during the test. The number of live bed bugs observed inside each apartment unit was recorded before each application (Table 1).

Visual counts were taken prior to the initial treatment in all units including the controls, and again at days 3, 5, 7, 14, 28, 42, and 56. Numbers of live bed bugs along the ceiling, walls, baseboards, cracks and crevices, mattresses and boxsprings, couches, nightstands, and chairs were recorded. Visual counts after day 7 were taken on the same day as the maintenance treatment, but prior to the application.

*Amount of formulation applied.* Treatment products were weighed in the application equipment before and after each application to determine the amount of product (in grams) applied in each apartment unit. Total product applied was calculated for both the Traditional and Novel treatments and then divided by the number of apartment units (5) to determine the average number of grams applied per product per apartment unit (Table 2).

**Statistical Analysis.** Visual counts were recorded by treatment (traditional, novel, and control) for each test day. The efficacy of a particular treatment, either novel or traditional, was determined using repeated measures ANOVA (SAS Institute 2005). The intent of this analysis was to compare the number of live bed bugs on a particular test day to the number recorded on all previous test days. The expectation was that the treatment combination(s) would result in a significant reduction in the mean number of live bed bugs by the end of the test period. Another feature of the repeated measures analysis was the inclusion of the baseline population measurement as a covariant. Since the apartment units had different levels of bed bug infestation prior to treatment, the untreated population means were adjusted to improve the homogeneity of the variances and the normality of the data. Differences between treatments (novel, traditional,

or control) were determined using the Tukey-Kramer test. Values of  $P \leq 0.05$  were used to indicate significance (SAS Institute 2005).

## Results

*Traditional treatment.* Prior to the application of the traditional treatment combination, the mean number of bed bugs observed in each apartment unit was 39.8 (Fig. 1). On day 3, the mean number of bed bugs recorded after the first treatment application had dropped to 5.6. The mean number of bed bugs recorded between day 7 and day 28 ranged from 5.2 to 10.6. However, bed bug counts dropped to 2.6 by day 42 and were further reduced to an average of 2.2 by the test termination date. In the traditional treatment combination, bed bugs were found on the walls, couches, and mattresses of the apartments (Table 1). By the end of the test, there was a 100% reduction in the number of bed bugs on the wall and couches. There was a 94% reduction in the number of bed bug on the mattresses in the traditional treatment units. Overall, the traditional treatment combination reduced the number of live bed bugs by an average of 95% over the eight week test period. The repeated measures analysis determined that the traditional treatment did significantly reduce the number of bed bugs from the pretreatment infestation levels ( $df = 6$ ,  $F = 2.79$ ,  $P = 0.02$ ).

*Novel treatment.* The average number of live bed bugs in apartment units selected for the novel treatment combination was 71.4 prior to the first application (Fig. 1). On day 3, the mean number of bed bugs recorded after the first treatment application had dropped to 20.8. By day 14, the mean number of bed bugs observed had risen to 31.8. Bed bug numbers declined to 16.2 by day 28 and continued to decrease to a mean of 10.2 by day 56. In the apartments receiving the novel treatment, bed bugs were only observed on the mattresses and walls. A 93% reduction in the mean number of bed bugs was observed on the mattresses. However, there was an 82%



reduction in the mean number of bed bugs observed on the walls. The novel treatment combination resulted in an 86% reduction in the number of bed bugs per unit at the end of the test. The repeated measures analysis determined that the novel treatment also significantly reduced the number of bed bugs from the pretreatment infestation levels ( $df = 6$ ,  $F = 2.68$ ,  $P = 0.02$ ).

*Control units.* The number of live bed bugs in the control units varied considerably from one monitoring period to the next due to our inability to gain consistent access to apartment units. At the initiation of the test, the mean number of bed bugs observed per apartment unit was 71.4 for all five apartments. On day 3, we could only gain access to one control apartment unit, which was heavily infested (113 bed bugs). The mean number of bed bugs recorded in four control apartment units on day 5 was 37.7. Access was denied on day 5 to the heavily infested apartment unit that we had recorded on day 3, lowering the overall mean number of bed bugs recorded. On day 7 we were again allowed access to the heavily infested unit, but denied access to the other four control apartment units. Therefore, the number of bed bugs recorded on day 7 was 165. On day 14 it was discovered that residents had thrown away a heavily infested bed frame, reducing the overall mean for control units to 18.3 bed bugs. On day 28 we were able to gain access to all five control units and the mean number of bed bugs was 26.6. On day 42, we had access to all five apartment units, however one resident was in the process of moving during the inspection. A mean number of 20.6 bed bugs were recorded on day 42. On the final day of the test, the mean number of bed bugs recorded was 19.7, however, we were only allowed access to 3 control apartment units. Therefore, we had to conclude that any observed reduction in control apartments was due to tenant behavior instead of any natural reduction in bed bug numbers ( $df = 6$ ,  $F = 3.71$ ,  $P = 0.003$ ).

*Comparison of treatment efficacy.* The ANOVA indicated that there was a significant treatment effect ( $df = 2$ ,  $F = 15.9$ ,  $P = 0.004$ ) and a significant time effect ( $df = 6$ ,  $F = 5.8$ ,  $P < 0.001$ ) on bed bug numbers. There was also a significant treatment time interaction ( $df = 12$ ,  $F = 1.9$ ,  $P = 0.05$ ). The Tukey-Kramer test indicated that the reduction in mean bed bug numbers in the traditional treatment was significantly greater from that of the control units ( $df = 12$ ,  $t = -5.7$ ,  $P = 0.0003$ ). The mean bed bug reduction in the novel treatment was also significantly greater than that of the control units ( $df = 12$ ,  $t = -3.5$ ,  $P = 0.01$ ). The novel and treatment combinations were not significantly different from each other at the  $\alpha = 0.05$  level, however the two treatment combinations were significantly different from each other at the  $\alpha = 0.10$  level ( $df = 12$ ,  $t = -2.4$ ,  $P = 0.08$ ).

*Product applied.* The mean amounts of formulated products applied for each treatment are listed in Table 2. The mean amount of total product applied in the traditional treatment was 5022.5 ml per apartment unit. However, this amount was not significantly different from the total mean amount of product in the novel treatment, which was 5220.9 ml per unit ( $df = 1$ ,  $F = 0.07$ ,  $P = 0.79$ ). There was significantly more active ingredient (193.1 mg) applied in the novel treatment apartments than in the traditional treatment apartments (122.5 mg) during the test period ( $df = 1$ ,  $F = 34.71$ ,  $P = 0.0004$ ).

## **Discussion**

The apartment units used in both treatment combinations were similar in size and floor plan. These floor plans were either one or two bedroom apartments with a living room and a possible dining room. The tenants that occupied these apartment units tended to live in large numbers (6-9), and were primarily male. The tenants would sleep in the bedrooms, the living

room, and in the dining room depending on how many tenants were occupying that particular unit. Some of the apartments had multiple beds in different rooms, while others did not have “beds” at all. Some tenants slept on stacked pieces of cardboard, balled up jackets or other pieces of clothing, while others slept directly on the floor. Because bedding varied considerably from apartment to apartment, the treatment of these locations was problematic. None of the products in either treatment combination were labeled for these “sleeping areas”. Products labeled for application to mattresses and boxsprings (Steri-Fab, N.I.C. 325, and Suspend SC) could not be applied to alternative beds. In addition, some apartments would be almost barren of belongings while other units were cluttered. Cluttered apartment units would have clothing piled in corners of the room or inside closets, making these areas difficult for inspection and treatment.

In spite of the difficult conditions, the results of this field test indicated that both the traditional and novel treatment combinations significantly reduced bed bug populations. While the traditional treatment was not statistically superior to the novel treatment, the absolute reduction in the number of bed bugs (95%) to a mean of 2.2 individuals per apartment not only reduced the presence of bed bugs, but also reduced the future infestation potential. While the novel treatment produced an 86% reduction, a mean of 10.2 bed bugs were still left in each apartment at the end of the test which greatly increased the likelihood of rapid reproduction and reinfestation. It should be noted that bed bug eggs were not quantified in either treatment.

In both the traditional and novel treatments, the greatest decrease in the number of bed bugs occurred after the initial treatment. The success of both treatment combinations during the first two counting periods (day 1-3) was a little surprising. When we tested a field strain of bed bugs in the laboratory we observed high levels of resistance to pyrethroids, especially to deltamethrin. We also observed an exceptionally high  $LT_{50}$  value (10 d 9 h) for chlorfenapyr.

However, those tests were conducted by confining bed bugs on dry residual deposits. The direct application of liquid insecticides directly to the insects, or contact to the wet residues increased the products' efficacy.

In the traditional treatment, the highest proportion of bed bugs were found on the ceiling/wall junction. Other population foci were located on the beds and other furniture. Direct application of pyrethroid insecticides on the bodies of the bed bugs as they aggregated on the beds and walls resulted in the large reduction observed on day 3. Subsequent maintenance treatments were applied in or on the same harborage sites. Frequently, live bed bugs were surviving after several treatments. It is possible that several individual bed bugs were exposed to multiple applications of pyrethroids during the course of this test. However, by the end of the eight week test period, bed bug populations were recorded in only three of the five pyrethroid treated units (3 to 4 bed bugs per apartment).

In the novel treatment, the highest proportion of bed bugs was located on the mattress and boxsprings. Other aggregations of the population were located on the walls and popcorn ceiling. The direct application of Steri-Fab on the bodies of bed bugs as they aggregated on bed seams during the initial treatment resulted in the large population reduction observed on day 3. For example, in one apartment unit 94 bed bugs were recorded on the bed prior to treatment. After the application of Steri-Fab, the number of bed bugs on the bed was reduced to 13. Direct application of Phantom to the walls of infested apartments also resulted in a 61% reduction in bed bug numbers by day 3. The quick knockdown activity of Phantom in the field was surprising because the laboratory assays presented in Chapter 3 indicated that Phantom was extremely slow acting ( $LT_{50}$  10 d 9 h). However, we believe that the direct application of the wet material to the bed bug bodies enhanced the cuticular penetration of all of our liquid

insecticide product, including pyrethroids. However, after the initial reduction in bed bug numbers, populations in the novel treatment remained the same (average of 22 per apartment) from day 3 to day 7. One of the reasons for this lag in activity was that Steri-Fab has no residual activity (Steri-Fab website 2005). A second reason was that the bed bugs were not susceptible to dry residual deposits of chlorfenapyr. Chlorfenapyr is a pro-insecticide and typically has to be ingested and metabolized to work (Van Leeuwen et al 2004). As stated above, chlorfenapyr may not penetrate the bed bug cuticle after the product has dried. Regardless of the reason, this lag period between days 3 and 7 allowed time for the surviving bed bugs to mate and reproduce. Therefore, we observed an increase in bed bug numbers on day 14. On day 14, a second application of Phantom was applied to the remaining bed bug aggregations on the walls and popcorn ceiling. N.I.C. 325 was also applied to the mattresses as a residual dust application. Both Phantom and N.I.C. 325 were applied every two weeks after the initial application for the remainder of the test period. After day 14, bed bug numbers on the walls were further reduced by the direct application of Phantom. Repeated applications of Phantom after day 14 continued to reduce bed bugs on the wall and ceilings until the termination of the test. Bed bugs treated with N.I.C. 325 were still active on the beds two weeks after the application (day 28) although they were covered with dust. However, by the end of the 8 week test period, bed bugs were only found on the bed in one apartment unit (9 bed bugs), indicating that N.I.C. 325 did control bed bugs, although it took a long time to work.

Access issues with control apartment units confounded the analysis of treatment efficacy in this field evaluation. Resident cooperation was very poor due to the fact that the research personnel were simply counting their bed bugs and making no effort to control them. When residents were home and did not observe any bed bug treatment efforts, they frequently denied

access for future inspections. Therefore inspections could only be made when residents were not at home and management provided access. One resident complained to treatment personnel about repeatedly inspecting her apartment but never applying any treatment. Another resident placed a sign on their door informing the research personnel that they would be applying their own bed bug treatments. Other residents threw out infested belongings, drastically reducing bed bug numbers in the control units. For example, a wooden bed frame was thrown out which reduced the mean number of bed bugs in that unit by almost 90% between day 7 and day 14, and lowering the overall bed bug numbers in the control units. The lack of cooperation on the part of the residents reduced our ability to accurately monitor control populations during the test period. The success of the treatment combinations was therefore difficult to determine simply by comparing treated bed bug numbers to control populations over time.

Even with the large amount of product (>5000 ml) applied and active ingredient used in the traditional and novel treatments, complete elimination of bed bugs did not occur. Multiple products and multiple applications were necessary for bed bug population reduction in multi-unit housing. Visual counts, though unreliable, were the only means of recording bed bug numbers in each apartment unit. However, bed bugs were still found in treated apartment units at the end of the test.

**Table 4.1.** Number of live bed bugs observed in each apartment unit by location.

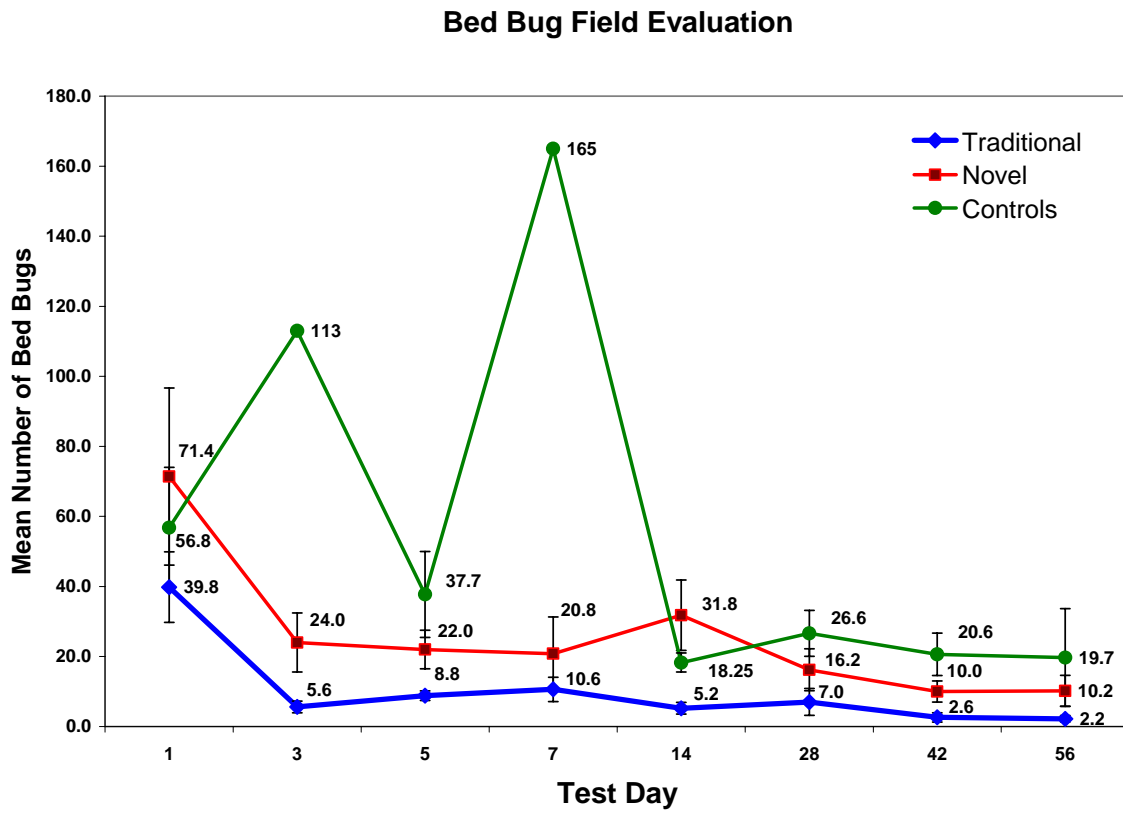
		Pre-treatment		Day 3		Day 56		
		number live		number live		number live		
Treatment	Bed bug	bed bugs		bed bugs		bed bugs		
Combination	location	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	% Reduction
Traditional								
N = 5	Mattress	1.8 $\pm$ 1.8	0 – 9	0.4 $\pm$ 0.4	0 – 2	0.0 $\pm$ 0.0	0 – 0	100
	Wall	36.4 $\pm$ 10.6	7 – 64	4.6 $\pm$ 1.9	1 – 10	2.2 $\pm$ 0.9	0 – 4	94
	Couch	1.6 $\pm$ 1.6	0 – 8	0.6 $\pm$ 0.6	0 – 3	0.0 $\pm$ 0.0	0 – 0	100
Novel								
N = 5	Mattress	24 $\pm$ 17.8	0 – 94	3.6 $\pm$ 2.5	0 – 13	1.8 $\pm$ 1.8	0 – 13	93
	Wall	45.4 $\pm$ 27.2	7 – 150	18.4 $\pm$ 7.4	4 – 46	8.4 $\pm$ 7.4	0 – 17	82

**Table 4.2.** Products applied in each treatment combination per unit per application.

Treatment Combination	Product	Mean amount (ml)	Mean amount (ml)	Milligrams A.I. per unit $\pm$ SE
		applied per unit $\pm$ SE	per unit per application $\pm$ SE	
Traditional  N = 5	Suspend SC (0.06%)	1564.4 $\pm$ 198.4	711.1 $\pm$ 49.59	0.94 $\pm$ 0.12
	Tempo SC Ultra (0.05%)	2893.2 $\pm$ 69.6	723.15 $\pm$ 17.47	1.45 $\pm$ 0.04
	Gentrol Aerosol (0.36%)	565.5 $\pm$ 10.4	141.39 $\pm$ 2.59	2.04 $\pm$ 0.04
	Total:	5023.1 ml	1575.8 ml	4.43 mg
Novel  N = 5	Steri-Fab	1076.6 $\pm$ 301.0	1076.6 $\pm$ 310.0	XXX
	N.I.C. 325	91.3 $\pm$ 0.6	30.6 $\pm$ 0.19	XXX
	Phantom (0.5%)	3593.2 $\pm$ 74.3	898.3 $\pm$ 18.58	17.96 $\pm$ 0.09
	Gentrol Aerosol (0.36%)	440.8 $\pm$ 5.9	110.2 $\pm$ 1.47	1.58 $\pm$ 0.02
	Total:	5201.6 ml $\pm$ 91.3 mg	2115.4 ml $\pm$ 30.6 mg	18.39 mg



**Figure 4.1:** This is a chart of the mean number of bed bugs observed on each different test date for each of the three treatment regimens.



## **Chapter Five**

### **Evaluation of Hydroprene on Bed Bug Mortality and Fecundity**

#### **Introduction**

The number of bed bug infestations within the U. S. has dramatically increased since 1990 (Potter 2006a), with insecticide resistance unknown, particularly pyrethroid resistance has been suspected as a potential cause for the increase in bed bug populations in different areas of the nation.. Therefore, alternative measures for bed bug control must be considered (Potter 2006a). Insect growth regulators (IGRs) have been used for controlling a multitude of insect pests in the urban environment, such as cockroaches (Atkinson et al. 1992), pharaoh ants (Oi et al. 2000), termites (Sheets et al. 2000), and fleas (Rust and Dryden 1997), and could aid in controlling pyrethroid resistant bed bug populations (Gentrol 2005).

Chitin synthesis inhibitors (CSIs) disrupt the normal molting process of insects by interfering with the insect's ability to produce new exoskeleton while molting (Ware and Whitacre 2004). Chitin synthesis inhibitors also prevent the formation or polymerization of chitin in insect exoskeletons (Miller 1994). Chitin synthesis inhibitors can work as a contact insecticide, but the majority of CSI products require ingestion (Ware and Whitacre 2004). Bed bug nymphs which are fed chitin synthesis inhibitors cannot molt properly and are killed during the molting process (Schal and Hamilton 1990).

The chitin synthesis inhibitors, such as hexaflumuron, diflubenzuron, and noviflumuron have been shown to control termites when delivered in bait systems (Su and Scheffrahn 1993, Karr et al 2004). Chitin synthesis inhibitors are passed from termite to termite by trophallaxis (Miller 2002). Because these CSIs are slow acting, the

trophallactic transfer from one termite to another allows for the entire colony to be affected.

Diflubenzuron is used to control a variety of lepidopteran insect pests and weevils (Dimilin 4L label 1997). A spray application, diflubenzuron has been very successful in aiding the Slow the Spread program for gypsy moths (Hoy 1982). Diflubenzuron is applied twice 7 – 14 days apart in isolated areas that are targeted for gypsy moths eradication (Dimilin 4L label 1997). Diflubenzuron kills gypsy moth larvae by interfering with the normal molting process, but has no effect on the adult gypsy moths.

Juvenile hormone analogs, the second type of IGRs, can be a very useful tool in controlling insect pests such as cockroaches, pharaoh ants, and fleas. Insect growth regulators (IGR) are compounds that affect the insect's ability to grow and mature properly (Ware and Whitacre 2004). Many insect growth regulators can either be classified as chitin synthesis inhibitors or juvenile hormone analogs (JHAs). Hormone juvenile analogues interfere with the growth process and prevent the insect from developing into an adult capable of reproducing (Schal and Hamilton 1990). Juvenile hormone analogs are effective at low doses and present a minimal risk to humans and pets (Mohandass et al. 2006). Unlike chitin synthesis inhibitors, juvenile hormone analogs can be used as a contact insecticide. Juvenile hormone analogs can extend the larval or nymphal stages of insects, prohibit the larval stage from pupating, or cause infertile adults (Ware and Whitacre 2004). Juvenile hormone analogs disrupt the insect molting process by acting on the endocrine or hormone system of insects (Olmstead and Leblanc 2003, Ware and Whitacre 2004). The effects of JHAs are primarily experienced in the insect's final molt, in which the newly molted adult maintains some of its nymphal

characteristics (Miller 1994). These mature, but JHA-affected insects are called adultoids and are sterile due to the juvenile hormone analog's effect (Miller 1994, Koehler and Patterson 1990). Juvenile hormone analogs can take up to several months to affect an insect population because they do not kill insects but stop reproduction so that the population dies by attrition (Ellsworth and Martinez-Carrillo 2001).

Methoprene is a juvenile hormone analog used for control of fleas and pharaoh ants (Rust and Dryden 1997, Lim and Lee 2005). Methoprene prolongs larval development and prevents flea larva from pupating (Rust and Dryden 1997). In pharaoh ants, methoprene sterilizes the queen and prevents the larvae from developing normally (Vail and Williams 1995).

Pyriproxifen (1.3%, Archer, Syngenta Crop protection, Greensboro, NC) is another juvenile hormone analog available for flea treatment. Molt delays have been shown in cat fleas larvae when exposed to 100 ppm of pyriproxifen (Zakson-Aiken et al 2000). Pyriproxifen is also used to control cockroaches. However, when German cockroaches were exposed to Archer at the label rate (pyriproxifen 1.3%), an increase in the population number was observed after 4 weeks (Ameen et al 2005).

Fenoxycarb, another juvenile hormone analog, can be used to control fire ants, fleas, mosquitoes, and cockroaches. Fenoxycarb interferes with the molting of larvae and prevents nymphs from becoming adults (Ware and Whitacre 2004).

Hydroprene, a juvenile hormone analog, is primarily used for control of cockroaches, but it is labeled for bed bug control as well (Gentrol label 2005). Cockroach nymphs treated with hydroprene develop into the adult stage, but are deformed and infertile. Hydroprene specifically affects the aedeagus of the male

cockroach so that it is unable to function properly for mating (King and Bennett 1991). Because both cockroaches and bed bugs are hemimetabolous insects, bed bugs exposed to hydroprene would be expected to respond similarly to cockroaches (Gentrol label 2005). Preventing bed bug reproduction would greatly enhance bed bug control efforts. By using hydroprene in combination with other products, a pest control operator could provide a quick kill for the majority of the population and then inhibit reproduction in the remaining bed bugs. The purpose of this study was to evaluate the effects of hydroprene on bed bugs, specifically hydroprene's ability to prolong bed bug development and to reduce adult fecundity.

## **Materials and Methods**

**Bed Bug Rearing.** A susceptible strain of bed bugs, *Cimex lectularius* (Linnaeus), was acquired from Dr. Harold Harlan of the National Pest Management Association in February 2005. Dr. Harlan maintained this colony of bed bugs for over 32 years (since 1973), feeding them on himself. The susceptible strain bed bugs were contained in glass jars (wide mouth Mason, 360 ml) fitted with a standard twist-off metal ring lid over a plastic mesh top to facilitate bed bug feeding. Inside the jar, cardboard (4 cm x 10 cm) had been folded accordion style and stood on end so that bed bugs were able to crawl up the cardboard and feed through the mesh top.

Bed bug colonies have been reared in the Dodson Urban Pest Management Laboratory and maintained similarly to the method described above. Bed bugs were fed by a human host once a week. Feeding took place by having a host place their forearm against the mesh top of the colony rearing jars. Each jar of bed bugs was allowed to feed

on the host for 30 minutes. Bed bug feeding was conducted as approved by the Virginia Tech Institutional Review Board (IRB #06-165). Between feedings, bed bugs in rearing jars were stored in a closed incubator at ~27°C, ~60% RH, and a photoperiod of 12:12 L:D. In February 2006, a field strain of bed bugs was collected from an apartment complex (Buckingham Village) in Arlington, VA. The rearing and feeding methods used to maintain the field strain bed bugs were the same as those described for the susceptible strain.

**Development Evaluation. IGR application.** Fisher brand filter paper (P-8 creped, Fisher Scientific, Pittsburg, PA 15275) was cut into 8.8 x 2.2 cm strips. A filter paper strip was then treated with 53 µl of Gentrol Concentrate (9 % Hydroprene; Wellmark International Schaumburg, IL) or water. Treatment consisted of three applications of 8.8 µl of hydroprene on the one side of the filter paper. The paper was allowed 1 h to dry before the opposite side was treated (Figure 1). The treated strip of filter paper and two non-treated strips of filter paper were then folded in half lengthwise and placed inside a vial (25 mm (O.D.) x 95 mm), Ward's Natural Science, Rochester, NY) so that their folded edges were in direct contact (Figure 2).

*Bed bugs.* Bed bug eggs were harvested from adult bed bugs that had been confined in Petri dishes. Eggs were collected and held in an environmental chamber (12:12, L:D, ~28°C, ~55% RH) until 200 first instars had hatched. To keep all bed bugs at the same developmental instar, bed bugs were fed on the same day so that they molted into third instars simultaneously.

One hundred third instar bed bugs were chosen for both the IGR and control treatments. The bed bugs were divided into groups of ten and each group was placed

inside a vial. The vials were then covered with a mesh fabric at the top and secured with a rubber band and parafilm. The vials were then placed in a Rubbermaid 12 U.S. qt “SnapToppers” clear storage box (Rubbermaid Home Products, Wooster, OH 44691). A Hobo data logger (Onset Computer, Bourne, MA 02532) was placed inside the Rubbermaid box to record temperature (78-79°C) and humidity (59-64%). A salt water solution was placed inside the box in order to regulate humidity (Figure 3). Temperature was maintained by keeping the vials inside of an environmental chamber during the test period.

Hydroprene exposed bed bugs and control bed bugs were fed on the same day at the same time every other week so that molting could be controlled. To prevent cross contamination, treated bed bugs and control bed bugs were fed on different arms. Effects from hydroprene exposure could be recorded during each instar by controlling bed bug feeding.

Third instar bed bugs were first exposed to the IGR on day 1. On day 7 they received their first post exposure blood meal. Bed bugs were then allowed 7 days to molt to their fourth instar. After molting, one piece of the non-treated filter paper was removed and replaced with a second piece of filter paper that had been treated with Gentrol at the label rate (day 14), thus doubling the number of molecules of juvenile hormone analog present in the vial. On day 21, bed bugs were fed again (5<sup>th</sup> instar) and allowed to molt to adulthood over a two week period. These adult bed bugs (day 28) were then allowed two additional weeks of hydroprene exposure (day 42). Adult mortality was recorded daily for the two week exposure period. Dead bed bugs were stored in a freezer and saved for further analysis.

**Adult Deformities.** After all tests were completed, adult bed bugs (alive and dead) were separated by sex into different groups: control, hydroprene exposed that died mid-molt, hydroprene exposed that died after partial scleritization. These groups were then inspected for morphological abnormalities using a scanning electron microscope.

Bed bug samples were twice washed for 15 min in 0.1 M Na cacodylate buffer. Post-fixation, bed bugs were washed with 1% OsO<sub>4</sub> in 0.1 Na cacodylate buffer for 1 hour. Bed bugs were then washed two more times for 10 min each in Na cacodylate buffer. Bed bugs were then dehydrated for 15 min in graded alcohol series (15 %, 30%, 50 %, 70 %, 95 %, 100 %). The bed bug samples were then critically dried (LADD critical point dryer, Ladd Research, Williston, VT). After drying, bed bug samples were coated with gold, because the dried bed bug samples were poor conductors of electricity (SPI sputter coater, Structure Probe, Inc. / SPI Supplies, West Chester, PA). The gold was used to eliminate image distortion due to charging. Bed bugs were then placed inside the scanning electron microscope (Zeiss EVO 40, Carl Zeiss SMT inc., Germany) and any deformities in male and female genitalia were recorded.

**Egg Production and Nymph Hatch.** Surviving adult hydroprene exposed and control bed bugs were paired (one male and one female) inside a Petri dish three hours after they were fed their first adult blood meal and then confined onto a hardboard panel for observation. Bed bugs were paired in the following combinations: treated males combined with treated females, treated males combined with control females, control males combined with treated females, and control males combined with control females. These different pairings were used to observe any potential fecundity differences between the different groups and cross-mated pairs. Each pairing had at least 6 replications. The



number of eggs produced in these pairings was recorded daily for 14 days. After egg production was completed, egg hatch and the number of surviving nymphs were recorded daily.

**Statistical Analysis.** Bed bug egg production and egg hatch was recorded by treatment combination for each test day. Differences in the mean number of eggs produced and egg hatch by each bed bug pairing were determined using ANOVA (SAS Institute 2005). Means were separated using Fisher's test of least significant differences. Values of  $P \leq 0.05$  were used to indicate significance (SAS Institute 2005).

## Results

**Development Evaluation.** *Control bed bugs.* A mean of 11% (1.1 bed bugs per replication  $\pm 0.47$ ) of the bed bug nymphs died between the third and fifth instar, with < 2% mortality recorded per week. All of the surviving bed bugs molted from the last instar to adulthood without additional mortality. As expected, no developmental abnormalities were observed during the nymphal instars within the controls.

*Hydroprene exposed bed bugs.* A mean of 14% (1.4  $\pm$  0.31 bed bugs per replication) of bed bug nymphs died between the third and fifth instar, with < 3 % mortality recorded per week. Dead bed bugs were examined for deformities. No developmental deformities were observed for hydroprene exposed bed bugs in any of the nymphal stages.

Of the 86 hydroprene exposed bed bugs which survived to the fifth instar, the only IGR induced mortality occurred during the final molt. After the fifth instar, 16% of the bed bugs died mid-molt between the 5<sup>th</sup> instar and adulthood. The bed bugs which

died mid-molt had hindgut rupturing through the exoskeleton between the thorax and abdomen. An additional eighteen percent of the adult bed bugs molted into adults, but did not completely sclerotize and died. Finally, fourteen percent of the adult bed bugs died after molting, and ingested their first adult blood meal. The anatomy of the bed bugs appeared to be normal, so the cause of death was unknown.

**Adult Deformities.** *Control bed bugs.* Scanning electron microscopy was used to observe any developmental deformities in the control adult bed bugs. As expected, there were no deformities observed in the control bed bugs (Figures 6 and 7).

*Treated bed bugs.* Multiple deformities were observed in the genitalia of the hydroprene treated bed bugs. Figure 8 is a SEM picture of the paramere of a male bed bug which had died after completely molting but not sclerotizing properly. The paramere is misshapen and deflated when compared to a normal male bed bug paramere (Figure 8). A second type of deformity was detected in the male bed bug genitalia. When compared to an untreated bed bug, the hydroprene exposed bed bug paramere was ~25% shorter (Figure 9). A normal bed bug paramere is between 400-500  $\mu\text{m}$  in length, while the hydroprene exposed bed bug's paramere was ~300  $\mu\text{m}$  in length. Both types of male genitalia deformities would not allow the bed bugs exposed to hydroprene to mate. Female bed bugs which have been exposed to hydroprene had genitalia deformities as well. An SEM picture was taken of a female which had been exposed to hydroprene (Figure 10). The gonapophyses are wrinkled and almost unrecognizable when compared to females which have not been exposed to hydroprene (Figure 10).

**Egg Production.** There were differences in the mean number of eggs produced in each of the different treatment groups (Table 1). The analysis of variance indicated

that the treated males mated with treated females laid significantly more eggs (mean of 15.3 eggs) than the control males and control females (mean of 7.9 eggs;  $df = 3$ ,  $F = 3.92$ ,  $P = 0.017$ ). However, the treated males mated with treated females was not significantly different than the treated males mated with control females.

**Nymph Hatch.** The mean number of eggs that hatched within the different treatment groups was also significantly different (Table 1). Each of the treatment groups had eggs hatch. The overall egg hatch for the treated males combined with treated females group was 94.5% ( $\pm 3.79$ ). The treated males and untreated females group had 86.3% ( $\pm 4.15$ ) of the eggs hatch. Ninety four percent ( $\pm 5.36$ ) of the eggs in the untreated males combined with treated females group hatched. In the untreated males and untreated females group, 93.6% ( $\pm 2.80$ ) of the eggs hatched. The analysis of variance determined that the mean egg hatch from treated males combined with treated females hatch was significantly higher than all other treatment combinations tested ( $df = 3$ ,  $F = 3.41$ ,  $P = 0.029$ ).

## Discussion

Bed bugs in the current study displayed a variety of responses to hydroprene exposure. Hydroprene exposure did not appear to delay molting during bed bug development. However, hydroprene did cause mortality to occur in some bed bugs during the final molt. Approximately 50 % of the bed bugs died during or after the final molt to adulthood. However, these bed bugs died at different stages in the molting process. Some died mid-molt, others died after molting but before complete scleritization,

and a third portion appeared to be completely sclerotized but died possibly due to some incomplete sclerotization of internal structures.

The results of this current study were similar to Todd's (2006), who evaluated the effects of hydroprene on bed bugs. Third instar bed bugs were exposed to hydroprene using methods similar to those used in this current study. Todd (2006) evaluated the effects of three concentrations of hydroprene on bed bugs, a low concentration (0.00136 g AI/ft<sup>2</sup>), a medium concentration (0.00669 g AI/ft<sup>2</sup>), and a high concentration (0.01487 g AI/ft<sup>2</sup>). Bed bugs were in constant contact with the hydroprene for 7 weeks until they had either died or molted into adults. At the end of the study, 72% (low concentration) – 98% (high concentration) of the bed bug nymphs had survived to adulthood. After molting to the adult stage, adult mortality ranged from 66% (low concentration) – 100% (high concentration) among the three concentrations. Todd's study (2006) reported many of these adults died mid molt with the hindgut rupturing through the exoskeleton between the thorax and abdomen. The same effects of adults dying mid molt with hindgut rupturing through the exoskeleton were observed in this current study.

Hydroprene appeared to have little effect on the reproductive capabilities of bed bugs in the current study. The adult hydroprene treated bed bugs were able to mate and reproduce with other treated bed bugs and with control bed bugs. In fact, when both parents were treated with hydroprene, egg production was significantly greater than when both parents were controls. The F1 generation bed bugs from this study were selected from each of the treatment groups specified in Table 1 and were fed to determine if they developed normally through their first molt. All of the first instar bed bugs molted and

developed into second instars. This F1 generation had no mortality during the first molt, indicating that these bed bugs are capable of developing normally.

Results from this study concurred with Todd's (2006) observations for his low-rate hydroprene concentration, where adult bed bugs actually survived the molting process. Todd (2006) mated these adult bed bugs (exposed to hydroprene during nymphal development) and was able to produce F1 progeny.

The ability of hydroprene treated bed bugs to produce eggs, both in this study and the Todd study (2006) was particularly interesting because other studies involving JHAs have demonstrated reductions in insect fecundity. For example, Koehler and Patterson (1985) found a high degree of sterility in German cockroaches exposed to hydroprene. Although hydroprene exposure did not appear to reduce fecundity in bed bugs, morphological changes did occur in bed bugs exposed to hydroprene.

Insect growth regulator resistance has been documented multiple times in mosquitoes (Cornel et al. 2002, Paul et al. 2006) and in house flies (Pimprikar and Georghiou 1979, Kristensen and Jespersen 2003). Bed bugs have been shown to have resistance to a number of juvenoids. Radwan and Sehnal (1983) observed strong IGR resistance in bed bugs to 18 different compounds. Bed bugs exposed to these compounds displayed different developmental anomalies. Radwan and Sehnal (1983) observed some morphological changes in the bed bug genitalia and enlarged wing lobes. Other effects ranged from bed bugs failing to completely molt during different instars, 5<sup>th</sup> instar bed bugs failing to molt into adults, bed bugs unwilling to feed, or bed bugs molting into "superlarvae." When these "superlarvae" were treated with a second dose of IGR, they molted into a second "superlarvae." Radwan and Sehnal (1983) concluded that

*C. lectularius* was very resistant to all the juvenoids tested and could not recommend any of the juvenoids for control.

Although there may be hydroprene resistance in bed bug populations, IGRs can still be a useful tool for bed bug management. Hydroprene does cause developmental defects, causing bed bug mortality during the final molt into adulthood, thus reducing bed bug numbers. Insect growth regulators most likely should be used in combination with other insecticides (Chapter Four) as part of an integrated pest management program for bed bugs.

**Table 5.1.** Comparison of mean number of eggs produced and hatched in each treatment group.

Treatment Group	N	Mean Number of Eggs	Mean Number of Eggs	Mean
		Produced ( $\pm$ SE)*	Hatched ( $\pm$ SE)*	% Hatch ( $\pm$ SE)*
Treated ♂/ Treated ♀	6	15.3 $\pm$ 2.87 a	14.3 $\pm$ 2.76 a	94.5 $\pm$ 3.79 a
Treated ♂/ Untreated ♀	8	11.5 $\pm$ 2.04 ab	9.9 $\pm$ 1.72 b	86.3 $\pm$ 4.15 a
Untreated ♂/ Treatment ♀	8	9.1 $\pm$ 1.26 b	8.8 $\pm$ 1.40 b	94.6 $\pm$ 5.36 a
Untreated ♂/ Untreated ♀	15	7.9 $\pm$ 0.86 b	7.5 $\pm$ 0.85 b	93.6 $\pm$ 2.80 a

\*Means followed by different letters are significantly different (SAS Institute 2003).

## **Figure Legend**

**Figure 5.1.** Diagram of hydroprene application to filter paper. page 92

**Figure 5.2.** Filter paper placement inside vial for bed bug exposure top view p. 92.

**Figure 5.3.** Placement of bed bugs in vials, Hobo (records temp. and R. H.), and salt water solution (humidity regulation) inside storage container. page 93

**Figure 5.4.** Image of a hydroprene exposed bed bug which died mid-molt between 5<sup>th</sup> instar and adult. page 94

**Figure 5.5.** Image of a hydroprene treated bed bug which died after completing its final molt. However, the bed bug did not completely sclerotized. The pale abdominal region indicates incomplete sclerotization. page 94

**Figure 5.6.** Scanning electron microscope (SEM) image of a normal male bed bug paramere. page 95

**Figure 5.7.** Scanning electron microscope (SEM) image of a normal female bed bug's genitalia. page 96

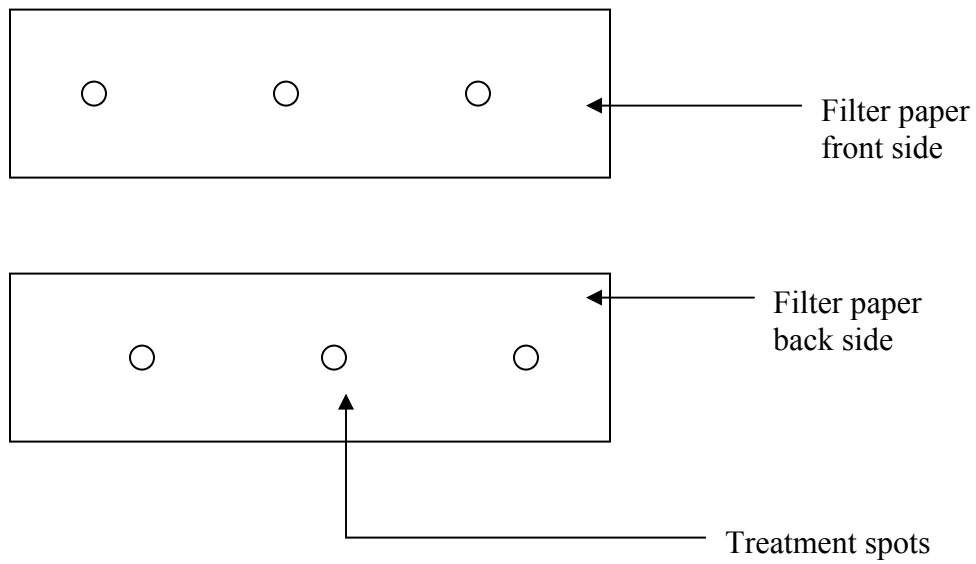
**Figure 5.8.** Scanning electron microscope (SEM) image of a hydroprene exposed male bed bug which molted into an adult, but did not completely sclerotize with the paramere deformed. page 97

**Figure 5.9.** Scanning electron microscope (SEM) image of a hydroprene exposed male bed bug which molted into an adult, but did not completely sclerotize. The paramere was 25% shorter than male controls page 98

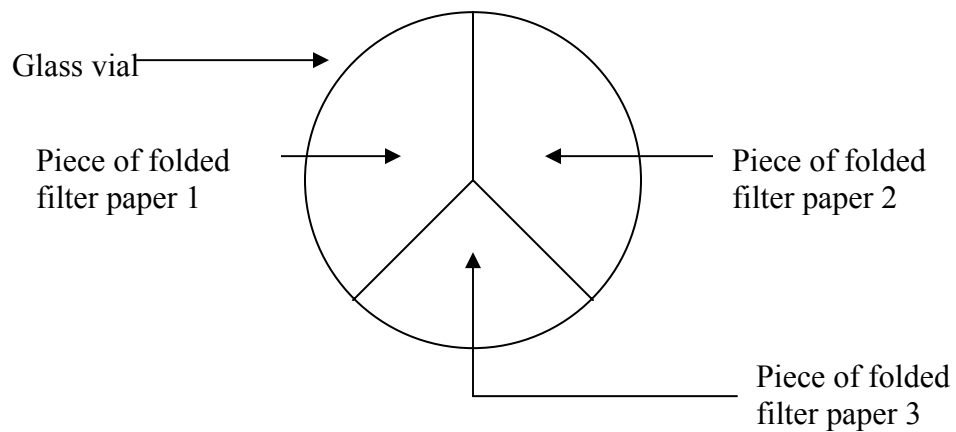
**Figure 5.10.** Scanning electron microscope (SEM) image of a hydroprene exposed female bed bug which molted into an adult, but did not completely sclerotize. The female genitalia was completely deformed. page 99



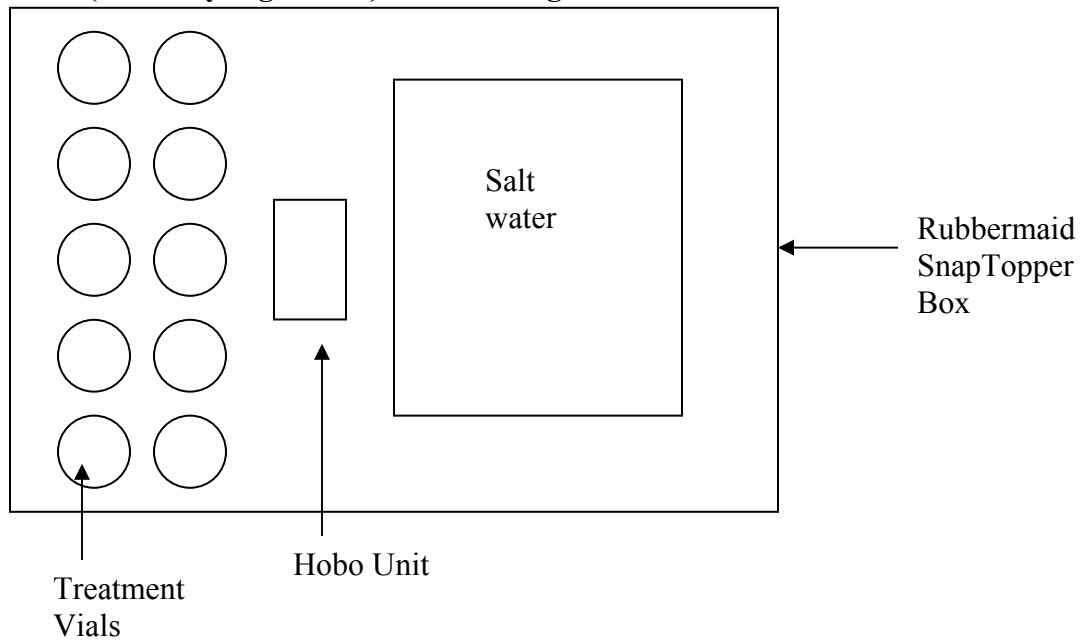
**Figure 5.1** Diagram of hydroprene application to filter paper.



**Figure 5.2** Filter paper placement inside vial for bed bug exposure top view.



**Figure 5.3** Placement of bed bugs in vials, Hobo (records temp. and R. H.), and salt water solution (humidity regulation) inside storage container.



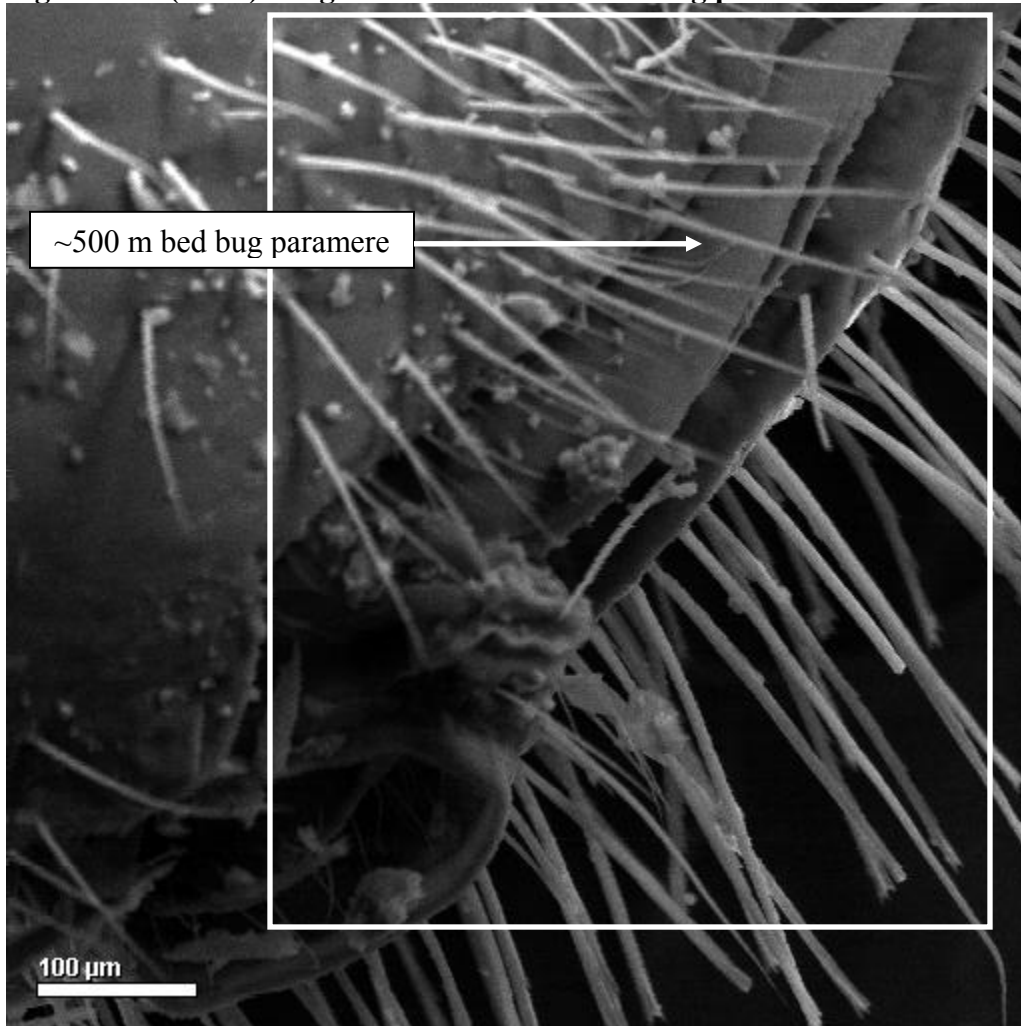
**Figure 5.4 Image of a hydroprene exposed bed bug which died mid-molt between 5th instar and adult.**



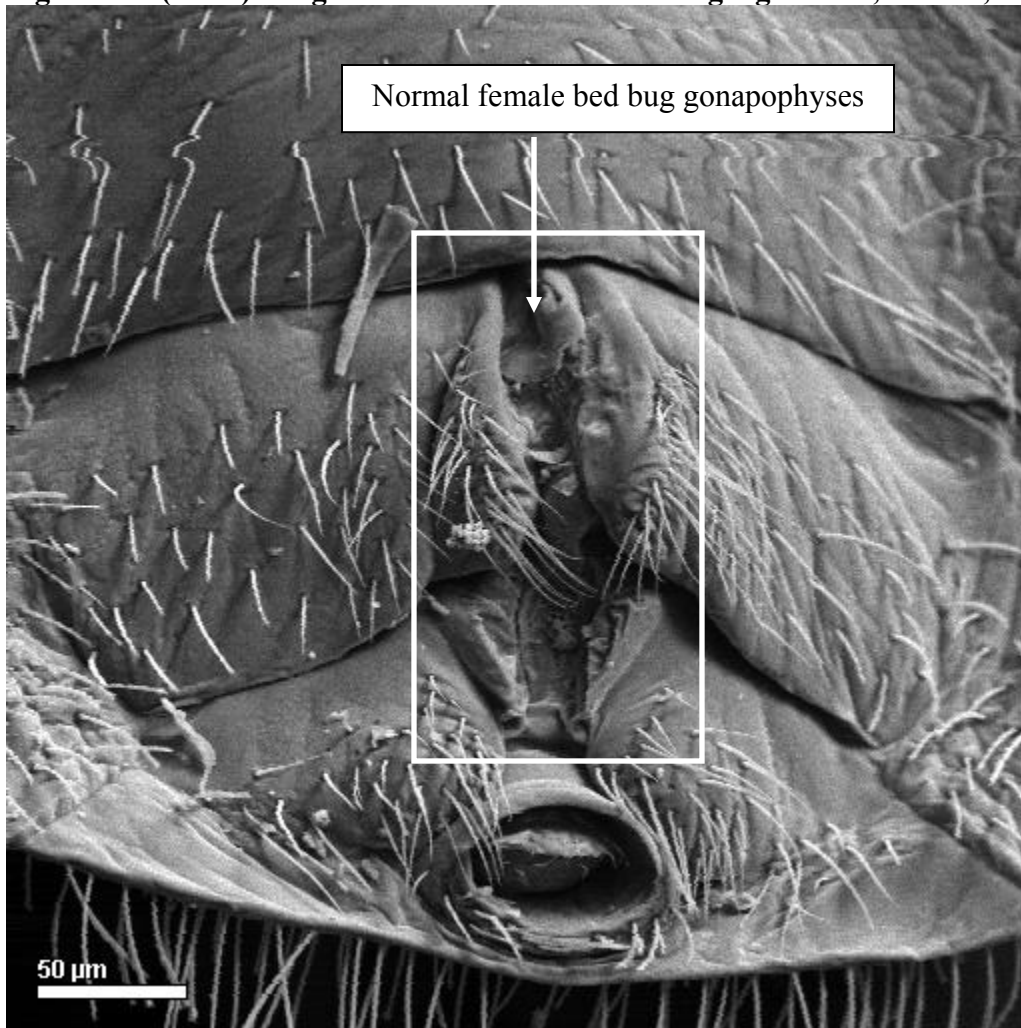
**Figure 5.5 Image of a hydroprene treated bed bug which died after completing its final molt.**



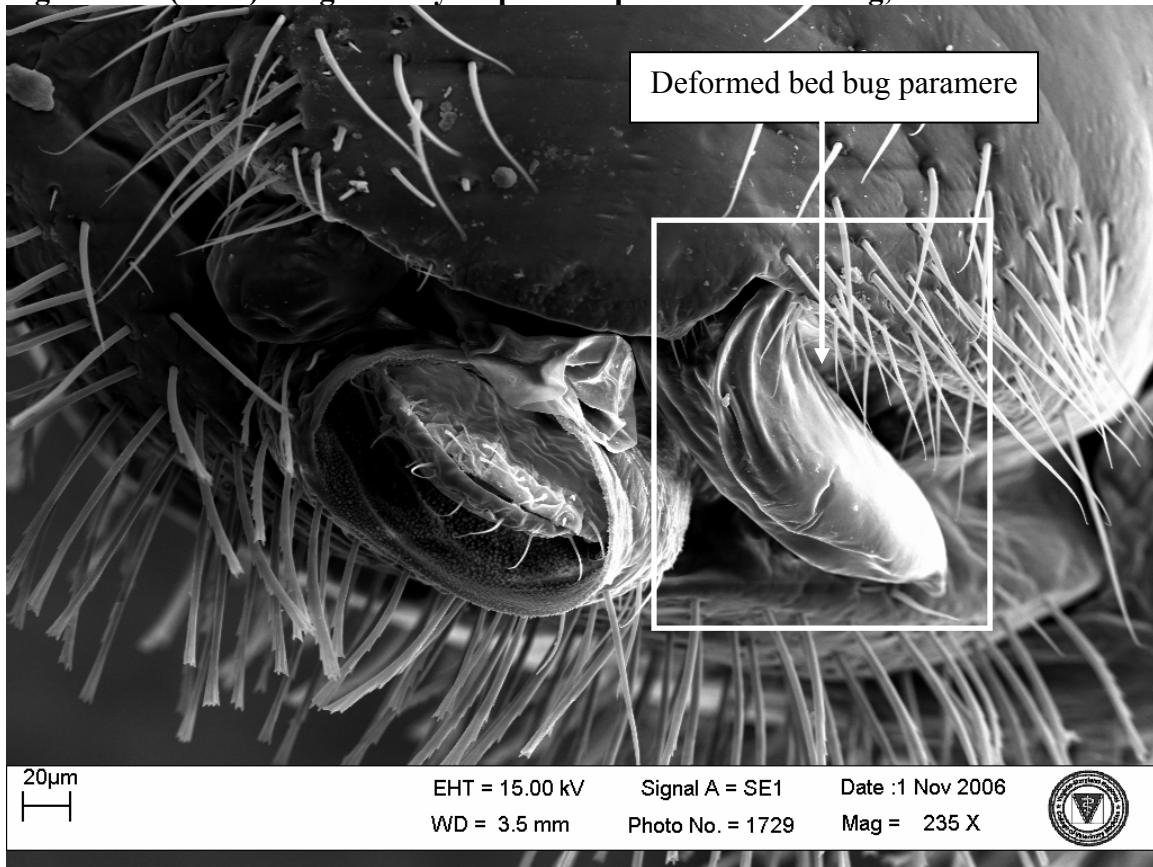
**Figure 5.6 (SEM) image of a normal male bed bug paramere**



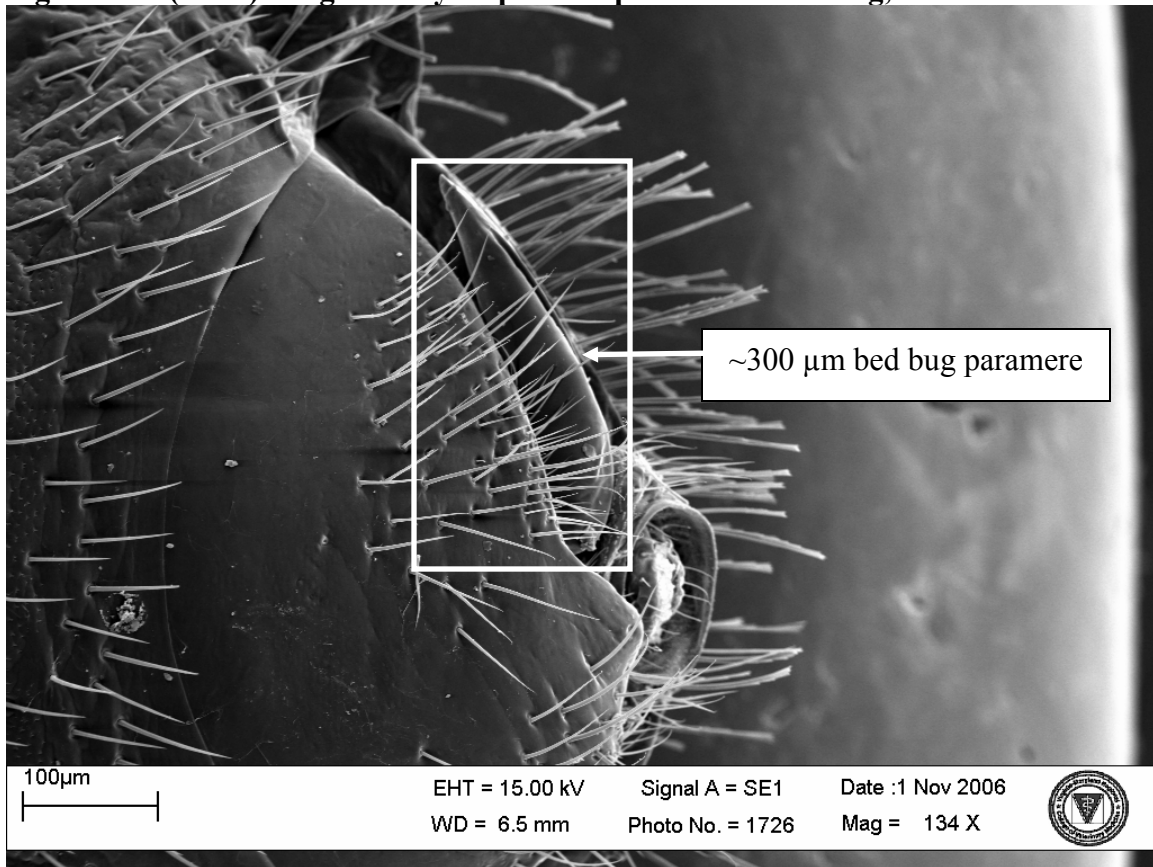
**Figure 5.7 (SEM) image of a normal female bed bug's genitalia, molted,**



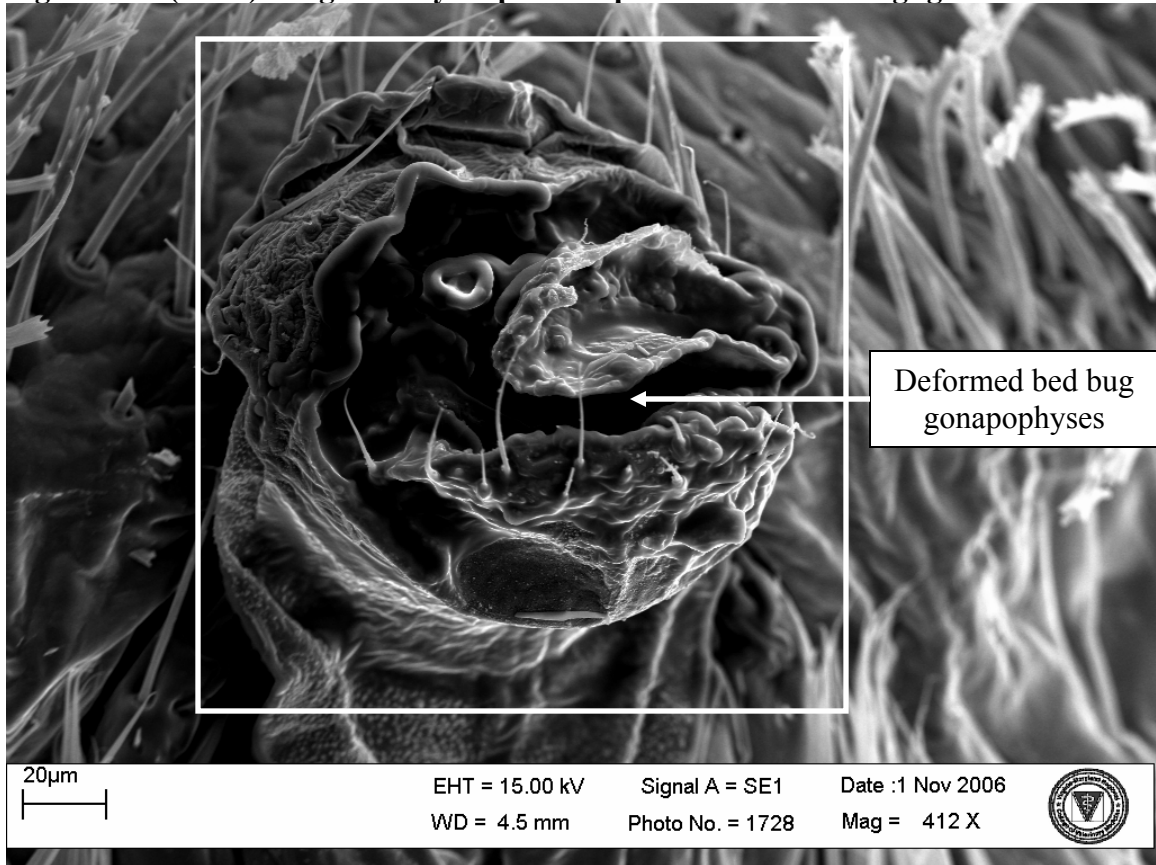
**Figure 5.8 (SEM) image of a hydroprene exposed male bed bug, molted**



**Figure 5.9 (SEM) image of a hydroprene exposed male bed bug, molted**



**Figure 5.10 (SEM) image of a hydroprene exposed female bed bug -genitalia deformed**





## **Chapter Six Summary**

Bed bugs have a high capacity for dispersal and are quickly becoming a widespread pest problem. However, proper bed bug management is in its infancy and pest management professionals are not adequately prepared to deal with this pest. The primary reason for this lack of preparation is that the pest control industry has no efficacy data for of the pesticide products currently being used for bed bug control. This situation is compounded by the fact that anecdotal information is being disseminated to the industry regarding the efficacy of “non-toxic” mattress treatments and the repellency of pyrethroid products. Therefore, the goal of this study was to determine the efficacy of the products being used for bed bug control so that quality recommendations can be made to the pest management industry about which commercial pesticide products actually work.

A susceptible, laboratory strain of the common bed bug was used to determine the efficacy of insecticide products labeled or possessing a site label for bed bug control. Field strain bed bugs were also used to evaluate pyrethroids. The  $LT_{50}$  values calculated for the field strain bed bugs and laboratory strain bed bugs exposed to the test products indicated that field strain bed bugs were significantly less susceptible to the pyrethroids than the laboratory strain. The  $LT_{50}$  value calculated for the field strain bed bugs exposed to deltamethrin was significantly greater than that of the laboratory strain bed bugs, with a resistance ratio of over 450. The resistance ratio for field strain bed bugs exposed to bifenthrin was 99, and the cyfluthrin  $LT_{50}$  value for the field strain bed bugs was 17 times greater than the calculated  $LT_{50}$  value for the susceptible strain bed bugs. The  $LT_{50}$  value for lambda-cyhalothrin in the field strain bed bugs was over 3 times greater and

significantly different from that of the susceptible laboratory strain bed bugs. However, a 3 fold increase in susceptibility is not classified as resistance. The  $LT_{50}$  values calculated on laboratory strain bed bugs for N.I.C. was 9 d 10 h. The fact that bed bugs are not readily susceptible to a non-insecticidal desiccant dust is not surprising when considering bed bugs can live for extended periods of time without feeding. Chlorfenapyr had an  $LT_{50}$  value of 10 d 9 h. Since chlorfenapyr is primarily a stomach toxicant, and bed bugs do not groom like other insects (such as cockroaches), these results indicate that chlorfenapyr should be used in combination with faster acting pyrethroids or as a resistance management tool. Both the N.I.C. 325 and chlorfenapyr  $LT_{50}$  values were higher than the pyrethroid products tested on the laboratory strain bed bugs. However, they were still able to kill bed bugs given enough time. This study suggests that while pyrethroids were effective for controlling laboratory strain bed bugs, there is significant resistance in field strains.

Surprisingly, none of the insecticides tested, including the pyrethroids, were repellent to laboratory and field strain bed bugs. Both laboratory and field strain bed bugs rested on pyrethroid treated panels and remained in contact with the panels until they died (2 h). This study also determined that pyrethroid products would not cause bed bug aggregations to scatter or avoid treated surfaces in the field.

Multiple product combinations (traditional and novel) were tested in an apartment complex for bed bug control. The traditional treatment consisted of products which are labeled for bed bug control and have been used for bed bug control in the past. The novel treatment consisted of newer chemistries which have not been evaluated for bed bug control. What was interesting was the drastic decrease in the bed bug populations after

the first application in both treatments. The traditional treatment combination reduced the number of live bed bugs by an average of 86% after the initial treatment. The novel treatment combination resulted in a 71% reduction in the number of bed bugs after the first application. Both the traditional and novel treatment combinations significantly reduced bed bug populations by the end of the test period. The decrease in bed bug numbers could be attributed to the direct application of the products to the bed bug bodies. These results indicated that direct applications to bed bug bodies had greater efficacy than when bed bugs in contact with dried insecticide residues tested in the laboratory. This greater bed bug efficacy was achieved by applying a mean amount of 5023.1 ml of product in the traditional treatment combination and mean of 5201.6 ml of product in the novel treatment combination. However, even after the large amounts of product were applied, bed bugs still persisted in most of the apartments.

Bed bugs were exposed to hydroprene in laboratory assays and the effects were recorded over a 7 week test period. Fifty percent of the bed bugs which survived to the fifth instar died during or after the final molt into adulthood. The exposed bed bugs which molted into adults successfully were then paired in different combinations (treated males combined with treated females, treated males combined with control females, control males combined with treated females, and control males treated combined with control females). All treatment groups were able to produce eggs. The treated males combined with treated females produced more eggs than other treatment pairs but did not produce significantly more eggs than the treated males combined with control females. Of the eggs laid, the treated males combined with treated females group had significantly more eggs hatch than all of the other treatment groups.

Overall, bed bugs are very difficult to kill. Many products took several days to kill laboratory strain bed bugs. Field strain bed bugs were found to be highly resistant to pyrethroids. Insect growth regulators did not effect bed bug nymphal development and killed only 50% of the pharate adults. Insect growth regulators did not reduce bed bug fecundity and actually increased egg hatch in exposed adults. With the research I have conducted, the pest control industry will have a better idea of how well bed bug control products work for bed bug management when the products are applied at the label rates.

## **List of References**

- Abbot, W.S. 1925.** A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.
- Ameen, A., C. Wang, W. Kaakeh, G. W. Bennett, J .E. King, L. L. Karr, and J. Xie. 2005.** Residual activity and population effects of noviflumuron for German cockroach (Dictyoptera: Blattellidae) control. J. Econ. Entomol. 98: 899-905.
- Adams, R. M. 1960.** The origins of cities. Sci. Amer. 203 :153-68.
- Atkinson, T. H. 1992.** Volatile effects of insect growth regulators against the German cockroach (Dictyoptera: Blattellidae). J. Med. Entomol. 29: 364-367.
- Bacot, A. W. 1914.** The influence of temperature, submersion and burial on eggs and larvae of *Cimex lectularius*. Bulletin of Entomological Research. 5: 111–117.
- Barkley, V. and P. C. Ellsworth. 2004.** Search for effective chemical controls for Lygus bugs and Whiteflies in Arizona. Arizona Cotton report (P-138). 187-205.
- Barnes, S. 1946.** The influence of certain biological factors on the resistance of bed bugs (*Cimex lectularius* L.) to DDT. Bull. Entomol. Res. 36: 419-22.
- Blow, J., M. Turell, A. Silverman, and E. Walker. 2001.** Stercorarial shedding and transtadial transmission of hepatitis B virus by common bed bugs (Hemiptera: Cimicidae). J. Med. Entomol. 38: 694–700.
- Boase, C. J. 2004.** Bed-bugs - reclaiming our cities. Biologist. 54: 1-4.
- Bodenheimer, F.S. 1929.** Materialien zur geschichte der entomologie bis linne. 2: 486.
- Brooks, S. E., F. M. Oi, and P. G. Koehler. 2003.** Ability of canine termite detectors to locate live termites and discriminate them from non-termite material. J. Econ. Entomol. 96: 1259-1266.

- Burton, G. J. 1963.** Bed bugs in relation to transmission of human diseases. Pub. Health Re. 78: 513-24.
- Burnett, J. W., G. J. Calton, and R. J. Morgan. 1986.** Bedbugs. Cutis. 38: 20.
- Busvine, J. R. 1959.** Insecticide-resistance in bed-bugs. Bull. World Health Organ. 19: 1041-1052.
- Busvine, J. R. 1980.** Insects and Hygiene: the Biology and Control of Insect Pests of Medical and Domestic Importance. 3rd edition. Chapman and Hall London, England: 230-240.
- Carayon, J. 1964.** Les aberrations sexuelles “Noralisees” de certains *Hemipteres Cimicoidea*. In A. Brion, et Henri Ey (eds.). Psychiatrie animale. Bibliotheque neuro- psychiatrique de langue Francaise.
- Christophers, S. R., and F.W. Cragg. 1922.** On the so-called “penis” of the bed bug (*Cimex lectularius* L.) and on the homologies generally of the male and female genitalia of this insect. Indian J. Med. Res. 9: 445-63.
- Cochran, D.G. 1993.** Decline in resistance in the absence of selection pressure in a population of German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 86: 1639-1644.
- Cooper, R. 2006.** Bed Bugs- still more questions than answers: a need for research and public awareness. American Entomologist. 5: 111-112.
- Cooper, R., and H. Harlan. 2004.** Ectoparasites, Part 3: bed bugs & kissing bugs, pp. 494-529. In S. Hedges (ed.). 9<sup>th</sup> ed., Mallis’ handbook of pest control. GIE Publishing, Cleveland, OH.

- Cornel, A. J., M. A. Stanich, R. D. McAbee, and F. S. Mulligan III. 2002.** High level methoprene resistance in the mosquito *Ochlerotatus nigromaculis* (Ludlow) in Central California. *Pest Management Science*. 58: 791-798.
- Cowan, F. 1865.** Curious facts in the history of insects. J.B. Lippincott & Co. Philadelphia. pp. 396.
- Craig, C. F., and F. C. Faust. 1970.** Clinical parasitology. 8th ed. Philadelphia, Pennsylvania.
- Davis, N. T. 1956.** The morphology and functional anatomy of the male and female reproductive systems of *Cimex lectularius* L. (Heteroptera, Cimicidae). *Ann. Entomol. Soc. Am.* 49: 466- 493.
- Dimilin 4L label. 1997.** Uniroyal Chemical Company, Inc. Middlebury, CT.
- Doggett, S., M. Geary, and R. Russell. 2004.** The resurgence of bed bugs in Australia: with notes on their ecology and control. *Environ. Health*. 4: 30–38.
- Dolling, W. R. 1991.** The Hemiptera. Oxford University Press, New York, New York.
- Ebeling, W., R. E. Wagner, and D. A. Reiersen. 1966.** Influence of repellency on the efficacy of blatticides. I. Learned modification of behavior of the German cockroach. *J. Econ. Entomol.* 59:1374-1388.
- Ebeling, W. 1975.** Urban Entomology. University of California, Los Angeles. 695 pp.
- Ellsworth, P.C. and J. L. Martinez-Carrillo. 2001.** IPM for *Bemisia tabaci*: a case study from North America. *Crop Protection* 20: 853–869.
- Elston, D. M., and S. Stockwell. 2000.** What's eating you? Bedbugs. *Cutis*. 65: 262-264
- Epps, S.B. 1968.** Through some eventful years. University of Florida Press, Gainesville, FL.

- Feroz, M. 1974.** The effect of additives on the toxicity of malathion and fenclorophos to a susceptible and an organophosphorus-resistant strain of the bed-bug, *Cimex lectularius* (L.) Biol. Pakistan. 20: 103-110.
- Foster, S. 1999.** Black Cohosh: *Cimicifuga racemosa*, a Literature Review. Herbalgram 45: 35-49.
- Fu, W.Z. and Y. K. Shong. 1995.** A clinical study of the relationship between bed bugs and allergic asthma. Chinese J. Vect. Biol. and Cont. 6: 54-57.
- Gaaboub, I.A. 1971.** Present status of DDT and dieldrin resistance in the bed-bug, *Cimex lectularius* in Alexandria district, United Arab Republic. Zeitschrift fuer Angewandte Entomologie. 68: 176-180.
- Gangloff-Kaufmann, J., C. Hollingsworth, J. Hahn, L. Hansen, B. Kard, and M. Waldvogel. 2006.** Bed Bugs in America: A pest management industry survey. American Entomologist. 52: 105-6.
- Gbakima, A. A. , B. C. Terry, F. Kanja, S. Kortequee , L. Dukuley , and F. Sahr. 2002.** High prevalence of bedbugs *Cimex hemipterus* and *Cimex lectularis* in camps for internally displaced persons in Freetown, Sierra Leone: a pilot humanitarian investigation. West Afr. J. Med. 21: 268-271.
- Gentrol IGR concentrate label. 2005.** Wellmark International. Schaumburg, IL
- Girault, A. A. 1912.** Preliminary studies on the biology of the bed bug, *Cimex lectularius*. II. Facts obtained concerning the duration of its different stages. J. Econ. Biol. 7: 163-188.
- Gooch, H. 2005.** Hidden profits: there's money to be made from bed bugs—if you know where to look. Pest Contr. Mag. 73: 26-32.



- Gunn, W.C. 1933.** The bed bug (*Cimex lectularius*): prevention of house infestation: a study for public health purposes. Department of Health for Scotland. His Majesty's Stationary Office, Edinburgh. p. 20.
- Hand, W.D. 1956.** Western Folklore 15: 290.
- Harlan, H. J. 2006a.** Bed Bug 101: the basics of *Cimex lectularius*. American Entomologist. 52: 99-101.
- Harlan, H.J. 2006b.** Bed Bugs - Importance, biology, and control strategies. Armed Forces Pest Management Board: Technical guide no. 44: 15.
- Hassan, S. A. 1985.** Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WORS Working Group: Pesticides and Beneficial Organisms. Bull. OEPP/EPPO 15: 214–255.
- Hase, A. 1917.** Ueber Lebensbedingungen, Verhalten und fruchtbarkeit der tropischen hauswanze *Cimex rotundatus* Sign. (Hem. Rhynch.) in Venezuela. Zeitschrift fur Parasitenkunde. 3: 837-893.
- Harves, A. D., and L. E. Millikan. 1975.** Current concepts of therapy and pathophysiology in arthropod bites and stings. Part 2. Insects. Int. J. Dermatol. 14: 621-634.
- Hect, O. 1930.** Die hautreaktion auf insektenstiche als allergische erscheinung. Zool. Anz. 87: 94-109, 145-157, 231-246.
- Horvath, G. 1914.** La distribution géographique des cimicides et l'origine des punaises des lit. IXe Int. Congr. Zool, Monaco. 294-299.

- Hoy, M.A. 1982.** Aerial dispersal and field efficacy of a genetically-improved strain of the spider mite predator *Metaseiulus occidentalis*, Acarina: Phytoseiidae. Entomol. Expt. Appl. 32: 205-212.
- Huntley, A. C. 1999.** *Cimex lectularius*. What is this insect and how does it affect man? Dermatol. Online J. 5.
- Hwang, S. W., T. J. Svoboda, I. J. De Jong, K. J. Kabasele, and E. Gogosis. 2005.** Bed bug infestations in an urban environment. Emerg. Infect. Dis. 1111: 533–538.
- Johnson, C.G. 1940.** Development, hatching and mortality of the eggs of *Cimex lectularius* L. (Hemiptera) in relation to climate, with observations on the effects of preconditioning to temperature. Parasitology 32: 127-173.
- Johnson, C.G. 1942.** The ecology of the bed-bug, *Cimex lectularius* L. in Britain. J. Hyg. 41: 345-461.
- Johnson, M. S. and A. J. Hill. 1948.** Partial resistance of a strain of bedbugs to DDT residual. Med. News Letter. 12: 26-28.
- Jones, S.C. 2004.** Ohio State University extension bed bug fact sheet. HYG-2105-04
- Jordan, H. F. and J. W. Schneider. 1997.** Papular urticaria: a histopathologic study of 30 patients. Am. J. Dermopath. 19: 119-126.
- Jupp, P.G., and S. F. Lyons. 1987.** Experimental assessment of bedbugs (*Cimex lectularius* and *Cimex hemipterus*) and mosquitos (*Aedes aegypti formosus*) as vectors of human immunodeficiency virus. AIDS 1:171-174.
- Karr, L. L., J. J. Sheets, J. E. King, and J. E. Dripps. 2004.** Laboratory performance and pharmacokinetics of the benzoylphenylurea noviflumuron in eastern

- subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 97: 593-600.
- Kells, S.A. 2006a.** Bed Bugs: a systemic pest within society. American Entomologist. 52: 107-108.
- Kells, S.A. 2006b.** Nonchemical control of bed bugs. American Entomologist. 52: 109-110.
- Kemper, H. 1930.** Beitrage zur biologie der bettwanze (*Cimex lectularius* L.). I. Ueber den einfluss des nahrungsmangels. Z. Morphol. Oekol. Tiere. 19: 160-83.
- Kemper, H. 1932.** Beitrage zur biologie der bettwanze (*Cimex lectularius* L.) ueber den einfluss des nahrungsmangels. Z. Morphol. Oekol. Tiere 24: 491-517.
- Kemper, H. 1936.** Die Bettwanze und ihre Bekampfung. Z. Kleintierk., Pelztierk. 12: 1-107.
- Kemper, H. 1959.** Die tierischen schadlinge im sprachgebrauch. Duncker und humblot, Berlin. 72-76, 152, 206, 367.
- Kettle, DS. 1995.** Medical and veterinary entomology, 2nd ed. Cambridge: UK. University Press.
- King, J. E. and G. W. Bennet. 1991.** Sensitive developmental period of last-instar German cockroaches (Dictyoptera: Blattellidae) to fenoxycarb and hydroprene. J. Med. Entomol. 28: 514-517.
- Knight, R.L. and M.K. Rust. 1990.** Repellency and efficacy of various insecticides against foraging workers in laboratory colonies of the Argentine ant, *Iridomyrmex humilis* (Mayr) (Hymenoptrera: Formicidae). J. Econ. Entomol. 83: 1402-1408.

- Koehler, P. G. and R. S. Patterson. 1989.** Effects of chitin synthesis inhibitors on German cockroaches (Orthoptera: Blattellidae) mortality and reproduction. J. Econ.Entomol. 82: 143-148.
- Kramer, R. D., P. G. Koehler, and R. S. Patterson. 1989.** Morphogenetics effects of hydroprene on German cockroaches (Orthoptera: Blattellidae). J. Econ. Entomol. 82: 163-170.
- Krinsky, W. L. 2002.** True bugs (Hemiptera). *In* Mullen G and L. Durden (eds.) Medical and veterinary entomology. Academic Press, Elsevier Science. 80-84.
- Kristensen, M. and J. B. Jespersen. 2003.** Larvicide resistance in *Musca domestica* (Diptera: Muscidae) populations in Denmark and establishment of resistant laboratory strains. J. Econ. Entomol. 96: 1300-1306.
- Krueger, L. 2000.** Don't get bitten by the resurgence of bed bugs. Pest Contr. Mag. 68: 58-64.
- Latimer, D. 2006.** Has pest control gone to the dogs? The PMP Reporter. Summer. pp 1, 8-9.
- Liebold, K., S. Schliemann-Willers, and U. Wollina. 2003.** Disseminated bullous eruption with systemic reaction caused by *Cimex lectularius*. J. Eur. Acad. Dermatol.Venereol. 17: 461-3.
- Lim, S. P. and C. Y. Lee. 2005.** Effects of juvenile hormone analogs on new reproductives and colony growth of Pharaoh ant (Hymenoptera: Formicidae). J. Econ. Entomol. 98: 2169-2175.
- Lyons, S. F., P. G. Jupp, and B. D. Schoub. 1986.** Survival of HIV in the common bedbug. Lancet. 5: 45.

- Mellanby, K. 1935.** A comparison of the physiology of the two species of bed-bug which attack man. *Parasitology*. 27: 419-428.
- Mellanby, K. 1939.** The physiology and activity of the bed bug (*Cimex lectularius* L.) in a natural infestation. *Parasitology*. 31: 200-211.
- Merton, T. 1955.** On a barrier against insect pests. *Proceedings of the Royal Society of London*. 218-220.
- Metschnikoff, E. 1887.** Ueber den phagocytenkampf beim ruckfalltyphus. *Virchow's Archiv Path. Anat. Physiol. Klin. Med.* 109: 176-92.
- Miller, D. M. 1994.** The integration of fecal extract (aggregaton pheremone) for use in German cockroaches (*Blattella germanica*) baiting strategies. Masters thesis. University of Florida.
- Mohandass S., F. H. Arthur, K. Y. Zhu, and J. E. Throne. 2006.** Hydroprene prolongs developmental time and increases mortality of indianmeal moth (Lepidoptera: Pyralidae) eggs. *J. Econ. Entomol.* 99: 1007-1016.
- Moore D. J. and D. M. Miller. 2006.** Laboratory evaluation of insecticide product efficacy for control of *Cimex lectularius*. *J. Econ. Entomol.* 99: 2080-2086.
- Moufet, T. 1634.** *Insectorum sive minimorum animalium theatrum*. Thom. Cotes, London. 18 + 326 p. (Transl. by E. Topsel, 1658 in “the History of Four-footed Beasts, Serpents and Insects.” E. Cotes., London. Chapter XXV on Wall Lice, p 1096-8).
- Newberry K. and E.J. Jansen. 1986.** The common bed bug *Cimex lectularius* in African huts. *Trans. R. Soc. Trop. Med. Hyg.* 80: 653-658.

- Nutall, G. H. F. 1900.** On the role of insects, arachnids and myriapods, as carriers in the spread of bacterial and parasitic disease of man and animal. A critical and historical study. John Hopkins Hospital Reports, Baltimore, Chapter 8: Cimicidae. 13-14, 17, 18, 20, 22-25, 32-33, 39, 120.
- Oakley, K.P. 1956.** Fire as a paleolithic tool and weapon. Proc. Prehistoric Soc. 21: 36-48.
- Oi, D. H., Vail K. M., and D. F. Williams. 2000.** Bait distribution among multiple colonies of phanroah ants (Hymenoptera: Formicida). J. Econ. Entomol. 93: 1247-1255.
- Olkowski, W., S. Daar, H. Olkowski. 1991.** Common sense pest control VII. Taunton Press, Newton, Conn. pp. 725.
- Olmstead, A. W., W. Allen, and G. A. Leblanc. 2003.** Insecticidal juvenile hormone analogs stimulate the production of male offspring in the crustacean *Daphnia magna*. Environmental Health Perspectives. 111: 919-924.
- Okey, T. 1930.** A basketful of memories. J. M. Dent & Sons Ltd. London & Toronto. pp. 128–129.
- Owen, T. 1806.** Geoponika: Agricultural pursuits. Thomas Owen. London, England.
- Paul, A., L., C. Harrington, and J. G. Scott. 2006.** Evaluation of novel insecticides for control of dengue vector *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 43: 55-60.
- Pimprikar, G. D. and G. P. Georghiou. 1979.** Mechanisms of resistance to diflubenzuron in the house fly, *Musca domestica* (L.). Pesticide Biochemistry and Physiology. 12: 10-22.

- Pipkin, A. C. Sr. 1969.** Transmission of *Trypanosoma cruzi* by arthropod vectors: anterior versus posterior route infection. *Int. Rev. Trop. Med.* 3: 1-47.
- Potter, M.F. 1997.** Invisible itches: Insect and non-insect causes. University of Kentucky Cooperative Extension Service, College of Agriculture. ENT- 58.
- Potter, M. F. 2004.** Your guide to bed bugs. *Pest Control Technology* Vol. 32 (8): [A special 6-page “pull out” section between pages 12 and 13 of the August 2004 issue].
- Potter, M. F. 2005.** A bed bug state of mind: emerging issues in bed bug management. *Pest Control Technol.* 33: 82-85, 88, 90, 92-93, 96-97.
- Potter, M. F. 2006a.** The Perfect storm: an extension view on bed bugs. *American Entomologist.* 52:102-104.
- Potter, M. F. 2006b.** Bed bugs exhibit resistance to pyrethroid insecticides, University of Kentucky Reports. *Pest Control Technol.* Online.  
<http://www.pctonline.com/news/news.asp?ID=4439>. accessed November 2 2006.
- Price, P. W. 1977.** General concepts on the evolutionary biology of parasites. *Evolution* 31: 405-420.
- Radwan, W. A., A. M. Guneidy, and A. A. Soliman. 1972.** On the biology of the bed bug, *Cimex lectularius* L. (Hemiptera, Cimicidae). *Bull. Soc. Ent. Egypte.* 56: 235-243.
- Radwan, W. A., and F. Sehnal. 1983.** Preliminary tests of juvenoids on the bed bug, *Cimex lectularius* L. *Bull. Soc. Ent. Egypte, Econ. Ser.* 11: 77-81.

- Rao, T. R., and A. V. Halgeri. 1956.** A note on resistance of bed bugs to DDT in Bombay State. *Indian J. Malariol.* 10: 149-154.
- Richardson, H. H. 1943.** The action of bean leaves against the bedbug. *In* H. Hartnack (ed.). *Unbidden house guests*. Hartnack Publishing Company, Tacoma, Washington, USA: 60–62.
- Rivney, E. 1930.** Host selection and cannibalism in the bed bug, *Cimex lectularius* L. *Ann. Entomol. Soc. Am.* 23: 758-764.
- Rivney, E. 1932.** Studies in tropism of the bed bug *Cimex lectularius* L. *Parasitology* 24: 121-136.
- Rivney, E. 1933.** The tropisms effecting copulation in the bed bug. *Psyche* 40: 115-120.
- Robertson, J. L., H. K. Preisler, and R. M. Russel. 2003.** PoloPlus: Probit and logit analysis user's guide. 36 pp.
- Rupp, W. J. 1946.** *Proc. Penn. German Soc.* 52: 266.
- Rust, M. K., and Dryden M. W., 1997.** The biology, ecology, and mangement of the cat flea. *Ann. Rev. Entomol.* 42: 451-473.
- Ryan, E. T., M. E. Wilson, and K. C. Kain. 2002.** Illness after international travel. *N. Engl. J. Med.* 347: 505-516.
- Sailer, R. I. 1952.** The bed bug. An old bedfellow that's still with us. *Pest Control*. 20 : 22, 24, 70, 72.
- Sansom, J. E., N. J. Reynolds, and R. D. Peachey. 1992.** Delayed reaction to bed bug bites. *Arch Dermatol.* 128: 272-273.
- SAS Institute. 2005.** SAS Institute, Inc., Cary, North Carolina.



- Scarupa, M. D. 2006.** Bedbug bites masquerading as uticaria. J. Allergy Clinic. Immunol. 117:1508-1509
- Schneemelcher, W. 1989.** New testament apocrypha 6<sup>th</sup> edition. Westminster/ Jones Knox Press. Louisville. Vol 2.
- Schal C. and R. L. Hamilton. 1990.** Integrated suppression of synanthropic cockroaches. Ann. Rev. Entomol. 35: 521-51.
- Schuh, R., and J. A. Slater. 1995.** True bugs of the world (Hemiptera : Heteroptera) classification and natural history. Cornell University Press, Ithica, New York.
- Sheets J. L., L. L. Karr, and J. E. Dripps. 2000.** Kinetics of uptake, clearance, transfer, and metabolism of hexaflumeron by eastern subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 93: 871-877.
- Silverman, A. L., L. H. Qu, J. Blow, I. M. Zitron, S. C. Gordon, and E. D. Walker. 2001.** Assessment of hepatitis B virus DNA and hepatitis C virus RNA in the common bedbug (*Cimex lectularius* L.) and kissing bug (*Rodnius prolixus*). Am. J. of Gastroenterology. 96: 2194.
- Sikes, E.K., and V.B. Wigglesworth. 1931.** The hatching of insects from the egg, and the appearance of air in the tracheal system. Quart. J. Microscop. Sci. 14: 165-192.
- Smith, V.K. 1979.** Improved techniques designed for screening candidate termiticides on soil in laboratories. J. Econ. Entomol. 72: 877-879.
- Smith, P. R., G. M. Lorenz, W. H. Robertson, D. Plunkett, D. R. Johnson, and R. Edmund. 2003.** Performance of diamond (Novaluron) for control of heliothines

- and plant bugs. Summary of Arkansas Cotton Report: AAES research series. 213-218.
- Snetsinger, R. 1997.** Bed bugs and other bugs, pp. 392–424. *In* S. Hedges ed. 9th ed., Mallis' handbook of pest control. GIE Publishing, Cleveland.
- Staal, G. B. 1986.** Anti juvenile hormone agents. *Ann. Rev. Entomol.* 31:391-429.
- Sterifab web site. 2005.** <http://www.sterifab.com/q%26a.html>. Accessed Oct 15 2006
- Su, N.-Y., and R. H. Scheffrahn. 1993.** Laboratory evaluation of two chitin synthesis inhibitors, hexaflumuron and diflubenzuron, as bait toxicants against the formosan subterranean termite and eastern subterranean termite (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 86: 1453–1457.
- Taylor, A. 1956.** *Folklore Studies.* 3: 29.
- Tharakaram, S. 1999.** Bullous eruption due to *Cimex lectularius*. *Clin. Experiment Derm.* 24: 241-242.
- Titschack, E. 1930.** Untersuchungen uber das Wachstum, den Nahrungsverbrauch und die Eirzeugung. 3 *Cimex lectularius* L. *Z. Morphol. Oekol. Tiere* 17: 471-551.
- Todd, R. G. 2006.** Efficacy of bed bug control products in lab bioassays: Do they make it past the starting gate. *American Entomologist.* 52: 113-115.
- Usinger, R. 1966.** Monograph of Cimicidae. Thomas Say Foundation, vol. 7 Entomological Society of America, College Park, MD. pp. 45.
- Vail, K. M., and D. F. Williams. 1995.** Pharaoh ant (Hymenoptera: Formicidae) colony development after consumption of pyriproxyfen baits. *J. Econ. Entomol.* 88: 1695-1702.

- Van Leeuwen, T., V. Stillatus, and L. Tirry. 2004.** Genetic analysis and cross-resistance spectrum of a laboratory-selected chlorfenapyr resistant strain of two-spotted spider mite (Acari: Tetranychidae). *Ex. and App. Acar.* 32: 249-261.
- Venkatachalam, P. S., and B. Beldavady. 1962.** Loss of haemoglobin iron due to excessive biting by bedbugs. *Transactions Royal Soc. Trop. Med. Hygiene.* 56: 218–221.
- Ware, G. W., and D. M. Whitacre. 2004.** *The Pesticide Book*, 6th Ed. Meister Media Worldwide, Willoughby, Ohio. 496 pp.
- Webb, P., C. Happ, G. Maupin, B. Johnson, and C. Ou. 1989.** Potential for insect transmission of HIV: experimental exposure of *Cimex hemipterus* and *Toxorhynchites amboinensis* to human immunodeficiency virus. *J. Infect. Dis.* 160: 970-977.
- Whitson, M., H. Harlan, and P. Koehler. 2001.** Bedbugs do bite, and they're back with a vengeance. *UniSci*, [WWW document]. URL <http://www.unisci.com> [accessed on September 12, 2006].
- WHO (World Health Organization). 1997.** *Vector Control Series*. Chapter 4. Bed bugs, fleas, lice, ticks, and mites: ectoparasites that live on the body, in clothes and in beds. World Health Organization. World Health Organization. Geneva, Switzerland. 237-243.
- Zakson-Aiken, M., L. M. Gregory, and W. L. Shoop. 2000.** Development of an assay for the screening of compounds against larvae of the cat flea (Siphonaptera: Pulicidae). *J. Med. Entomol.* 37: 571-574.