Life history of the common bed bug (*Cimex lectularius* L.) in the U.S.

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ABSTRACT

This study quantifies the rate of bed bug nymphal development, mortality, fecundity and survivorship during starvation for wild caught resistant populations. I then compare some of these characteristics with two susceptible strains. I found that resistant populations develop faster and exhibit less mortality per life stage than susceptible populations. However, there were no significant differences in the total number of eggs produced by the resistant females from the field strains during the 13 feedings/oviposistion cycles ($P = 0.106$). On average, resistant females from the field strains produced 0.74 eggs per day. Susceptible strains survived a significantly longer time without feeding (89.2 d and 81.4 d) than the resistant strains (RR, ER). The mean duration of adult life (from the day the female becomes an adult until the day she dies) for (RR) strains was 118.7 d ± 11.8 SE. The intrinsic rate of increase *r* or average daily output of daughter eggs by female was 0.42. The net reproductive rate R_o, indicated that one live female egg would, on the average, be replaced by approximately 35 females. Resistant and susceptible populations were found to be different in terms of development, survivorship, and fecundity. The differences between susceptible and resistant strains could be explained by a trade-off between the alleles that confer resistance and the fitness in the population. When compare the stable age distribution of a pyrethroid susceptible strain (HS) and a resistant strain (RR) there were not significant differences ($\chi 2 = 9.0066$, df = 6, $P = 0.1732$) in the stable age distribution, basically both strains were dominated by the egg stage. No significant difference was found in the expected reproductive contribution of the various life stages to future population size between the two strains ($\chi 2 = 1.5458$, df = 6, $P = 0.9564$). Despite this, the reproductive contributions of life

stages other than eggs were generally higher for the HS strain than for the RR strain. For both strains changes in P_i for the adult stage are expected to have the greatest impact on λ_m compared with changes in P_i for the other life stages. The key to the reduction of the populations of bed bugs lies with the reduction of survival of the adults.

Dedication

To Jucho

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Chapter 1: Introduction

The common bed bug, *Cimex lectularius* (L.), is a blood-feeding parasite of several mammals and birds; however, humans are the preferred host of this insect and the human environment provides optimal conditions for the bed bug's development. Three species of bed bugs are closely associated with humans, *Cimex lectularius, C. hemipterus* (F.), and *Leptocimex boueti* (B.) (Harlan et al. 2008). *C. lectularius*, known as "the common bed bug" is found in temperate regions and in any place where humans provide a favorable microclimate, food and shelter (Harlan 2007). Bed bugs take a blood meal approximately every 3-7 days if the host is available (Usinger 1966). According to Johnson (1941) adult female bed bug fed on rabbit blood can lay 14 eggs per week at room temperature at 23°C, 75% RH. Eggs hatch within seven to nine days. The developmental period from egg to adult is approximately five weeks if the immature bed bug takes a blood meal every week (Lehane 1991). However, the hatching and molting to the next life stage are influenced by temperature, which can speed up or prolong the developmental process (Johnson 1941, Robinson 2005).

Bed bugs have two important biological adaptations: they are obligate hematophagus insects, and they mate through traumatic insemination. Unlike mosquitoes, all of the bed bug's immature stages feed on blood. Immature bed bugs require this blood meal to molt to the subsequent instar. Both sexes of the adults also feed on blood in order to reproduce. Once the nutrition from a blood meal is exhausted, adults must feed again to produce additional eggs (Usinger 1966). Female bed bugs possess a genital tract, however, male bed bugs never use the female genital tract for insemination. Instead, male bed bugs pierce the female's abdomen with their perimere, inseminating her through her body wall (Usinger 1966). Females are sometimes

traumatically inseminated as many as five times per feeding, this is often sometimes 20 times more than necessary for fertilization to occur (Stutt and Siva-Jothy 2001).

Bed bugs are nocturnal insects that feed at night and hide in harborages by day. Bed bug harborages often contain exuvia, eggs, feces and individuals from different developmental stages. Generally, bed bugs prefer to establish their harborages near the host, and in enclosed spaces, such as, cracks, crevices, mattress seams and in the box springs (Usinger 1966). Enclosed spaces help them maintain an optimal microclimate for bed bug development. Bed bugs emerge from also like rough surfaces onto which they can walk comfortably and cement their eggs. Bed bugs feed at night when the host is asleep. Bed bugs typically feed from 5 to 20 minutes. Once they are fully engorged they leave the host to avoid injuries (Harlan 2006).

Bed bug bites cause human physical and psychological discomfort; however, disease transmittion has never been documented in the scientific literature. HIV and hepatitis B virus (HBV) have been studied extensively with regard to the potential to be transmitted by bed bugs. Jupp et al. (1978) and Webb et al. (1989) demonstrated that the HIV and the HBV viruses do not multiply inside of *C. hemipterus* or *C. lectularius'* gut and therefore, bed bugs are unable to transmit these diseases. However, bed bug bites can still produce itchy reactions and redness. In severe cases, bites can lead to secondary infections (Boase 2001). Bed bug infestations can also cause high levels of anxiety and stress among the people suffering from the bites (Boase 2001, Potter 2005).

Bed bugs were a common household pest in the U.S. at the turn of the 20th century. Bed bugs were transported in belongings and on public transportation. Bed bug infestations were common in hospitals, movie theaters, schools, dressing rooms and factories. The most popular

insecticides used to control bed bugs at the time were arsenic and mercury compounds, gasoline, and kerosene (Potter 2008).

Bed bug infestations were almost eradicated in the 1940s and 1950s due to the widespread application of DDT and organophosphate insecticides (Potter 2008). After 1942, DDT became "the perfect answer to the bed bug problem" (USDA Bureau of Entomology 1945). However, resistance to DDT was documented within 8 years of its initial use (Potter 2008). DDT was not the only insecticide to which bed bugs became resistant. Over the next several decades (1950s, 60s and 70s) bed bug populations in the U.S. and other nations developed resistance to a number of chemicals, most of which are no longer registered for bed bug control in the U.S. (Harlan 2006).

Starting in the 1990s bed bug infestations began to increase in the United States. The exact causes are unknown. As of mid-2008, bed bug incidences had been reported in all 50 states. The causes of the bed bug resurgence can be attributed to the increase in international travel (Boase 2001), human immigration from other countries (Cooper 2006, Potter 2005), seasonal human migration (Kells 2006), misidentification of bed bugs (Cooper 2006), and resistance to insecticides such as pyrethroids (Potter 2006).

Most of the older chemistries for bed bug control (carbamates and organophosphates) are no longer registered with the U.S. Environmental Protection Agency for indoor use. In fact, pyrethroid products are the primary insecticides used for bed bug control. The constant use of the same pyrethroids over time has led to pyrethroid resistance in bed bugs. Pyrethroid resistance has been well documented in *C. lectularius* (Moore and Miller 2006, Romero et al. 2007, Kyong et al. 2008) and in *C. hemipterus* (Karunaratne et al. 2007). As of 2010, pyrethorids are still the most widely used insecticides for bed bug control in the U.S. (Potter 2010).

Very little is known about the ecology and growth potential of modern day resistant bed bug populations. The most comprehensive work on bed bug ecology was conducted almost 70 years ago (Johnson 1941). However, the environment of modern bed bug infestations is quite different from that of populations 70 years ago. Bed bugs living in the 1930s were most likely limited in their development and reproduction by low indoor temperatures during the winter months. There was also no insecticide resistance documented in these 1930s populations. Therefore, we have very little information on the bed bug ecology and population dynamics that is relevant to the infestations proliferating in the U.S. today. There is a great need for ecological and biological studies of the species to understand the life history of bed bug populations in the modern indoor environments within the U.S. (Reinhardt and Siva-Jothy 2007).

The goals and objectives of this research project were:

1) To evaluate the lifelong egg production potential of three bed bug field populations during a period of 13 blood meals/oviposition cycles.

1a) To compare the fecundity between adult female bed bugs mated once versus mated multiple times for one field strain.

2) To determine bed bug survival during starvation for both nymphal and adult life stages (two resistant and two susceptible populations).

3) To develop life tables for the common bed bug (*Cimex lectularius*) under laboratory conditions for three populations (one laboratory-susceptible and two field-resistant populations).

4) To develop a stage-based projection model to assess and compare the structure of three bed bug populations (one laboratory-susceptible and one field-resistant populations).

Chapter 2: Literature Review

Classification and description of *C. lectularius*

Cimex lectularius belongs to the family Cimicidae in the order Hemiptera. The family Cimicidae contains 74 species, 22 genera and six subfamilies. Twelve of these genera feed on bats, while nine feed on birds (Usinger 1966). Only three species are closely associated with humans, *Cimex lectularius, C. hemipterus,* and *Leptocimex boueti* (Harlan et al. 2008). *C. lectularius*, known as "the common bed bug" is found in temperate regions, while *C. hemipterus* known as "the tropical bed bug" requires higher average temperatures and is found in the tropics and Florida (Harlan 2007). *L. boueti* also requires high temperatures for normal development, but its distribution is restricted to tropical West Africa (Lehane 1991). Other species of Cimicidaes will temporarily feed on humans when their primary host is absent. The poultry bug (*Haematosiphon inodorus* D.), pigeon bug (*C. columbarius* J.), eastern and western bat bug (*C. adjunctus* B.*, C. pilosellus* H.), chimney swift bug (*Cimexopsis nyctalis* L.) and two species of swallow bugs (*Oeciacius hirundinos* L. and *O. vicarious* H.) are some species of Cimicidae present in U.S. that may alternatively feed on humans when their natural host (birds and bats) are absent (Pinto et al. 2007).

Like all members of the order Hemiptera, bed bugs are hemimetabolous insects. Hemimetabolous insects have an incomplete metamorphosis. Hemimetabolous development has three distinct developmental stages: egg, nymph and adults. Nymphs resemble adults as they develop and there are not conspicuous differences between instars (Triplehorn and Johnson 2005).

The bed bug can be identified by a number of morphological features. The color of an adult bed bug may vary from brown to reddish-brown. Immatures are usually whitish to cream

colored. The coloration may vary depending on whether there is blood present in the gut or not (Robinson 2005). Adults range in size from 4 - 6 mm, are oval shaped and dorsoventrally flattened (same as top to bottom). The body is completely covered with minute setae arranged in bands. The front wings are vestigial and hind wings are absent. Bed bugs have a four segmented antennae, as well as a three segmented proboscis which is folded beneath the thorax and head when not being used for feeding. Eggs are 1 mm long, white, elongate-oval with the anterior end or cap exposed to allow for eclosion of the nymph (Usinger 1966).

A sweetish odor emitted from last thoracic segment glands is also a characteristic of bed bugs. It has been described as “sweet”, “musty” and “strawberry-like”. It is easy to detect when bed bugs are disturbed.

Origin of *C. lectularius* infestations

An archeological record has shown that bed bugs were co-habitating with humans 3,500 years ago (Lehane 1991). Bed bugs may have originally been ectoparasites of bats. The fact that a species of Cimicidaes, *Leptocimex boueti,* feeds on blood from bats and humans, suggests that the host-parasitoid relationship may have arisen during a period when humans occupied caves (Robinson 1996). From caves to villages, bed bugs moved with their human hosts and adapted to their living conditions. Humans provide the perfect microclimate for bed bug proliferation (Harlan 2007). Usually, comfortable human conditions are ideal for bed bug development (Robinson 1996).

Bed bug populations were common from the ancient civilizations to the new world. Bed bugs were actually popular in Greeks and Romans civilizations, not because of their annoying bites, but because the Greek and Roman believed bed bug potions could cure diseases and give

good luck. Bed bugs were repeatedly transported by humans into Europe and Asia from 100 A.D into the 1400's. Bed bugs were brought from Europe to America by the first settlers and were subsequently transported across the continent (Potter 2008).

Dispersion and eradication of *C. lectularius*

Before World War II, bed bugs were a very common household pest in Europe and America. Bed bugs were transported by humans in different modes of public transportation and belongings. Bed bugs were commonly found in laundries, dressing rooms, factories, schools, trains, buses, airplanes, hospitals and even in military installations. Overcrowded and low income communities were the most affected by bed bug infestations (Potter 2006). During 1930's and 40's bed bug infestations became as common as cockroaches and rats in European and American countries (Potter 2006).

Populations of bed bugs were mostly eradicated after World War II because of the development and widespread application of new synthetic insecticides. The synthetic insecticide, DDT (dichlorodiphenyl trichloroethane) greatly contributed to the elimination of bed bug populations in the U.S (Harlan 2006, Cooper 2006, Harlan 2007, and Potter 2008). Bed bug populations were nearly eliminated from most industrialized nations due to the use of DDT and malathion. In fact, bed bugs became so rare in developed countries, that only a few researchers kept bed bugs in the laboratory (Harlan 2006). However, some undeveloped countries were not successful in eliminating bed bug populations and have continued to support significant populations even into the 21st century (Harlan et al. 2008).

Reproduction in *C. lectularius*

C. lectularius has an unusual method of copulation called "traumatic insemination" (Usinger 1966). During this process, the male uses his reproductive organ, the paramere, to puncture the female directly in her abdominal cavity. During copulation the male paramere causes cuticular wounds in the female's abdomen. Cuticular wounds allow the male to deposit his sperm into the female's Organ of Berlese instead of her genital opening. The Organ of Berlese is a specialized organ located at the female's fifth abdominal sternite that serves as a storage organ for the male's sperm. Once inside the Organ of Berlese, the sperm migrates through the female's hemolymph until it reaches the ovaries where fertilization occurs (Usinger 1966).

Females can mate multiple times with multiple males, but each mating event causes integumental wounds that are costly to the females (Siva-Jothy and Stutt 2003). Females spend a lot of their energy healing the wounds produced by the traumatic insemination. Females also use significant amounts of energy fighting potential infections from introduced pathogens on the male's sexual organ (Reinhardt et al. 2003). Traumatic insemination can reduce the female bed bug's fitness in terms of life expectancy and egg production (Siva-Jothy 2006, Reinhardt and Siva-Jothy 2007, Hosken et al. 2009). Stutt and Siva-Jothy (2001) documented that bed bug females were typically mated 20 times more than required for fertilization. The physiological cost of multiple matings was quantified, and it was found that females exposed to large numbers of matings produced fewer eggs and have a shorter life-span than females exposed to a minimum number of matings (Stutt and Siva-Jothy 2001).

Egg production in *C. lectularius*

The female egg production cycle after a blood meal lasts approximately 12 days. Each female produces 6-10 eggs (23ºC) after taking a single blood meal. Johnson (1941) followed the egg production of female bed bugs and observed that after a blood meal, females started laying eggs on the 3rd - 5th day. Females continued laying eggs for eight to ten more days. If the females were fed again, they continued to lay eggs until the blood meal was exhausted. On average, a female bed bug was able to continuously lay eggs for 20 weeks when fed every week. The number of eggs laid was correlated with the amount of blood taken and with the size of the females. The larger the female, the larger the blood meal and consequently, the larger the number of eggs produced (Johnson 1940). Females typically produced fertile eggs, but they also produced infertile eggs that only partially developed and did not hatch. In addition females produced some sterile eggs that were not fertilized (Johnson 1941)

C. lectularius feeding behavior and bites

All bed bug developmental stages (except eggs), and both sexes, feed on blood (typically human blood) (Usinger 1966). To access the blood, bed bugs use their fascicle to pierce the skin of the host (Johnson 1941, Usinger 1966). With a fast movement, the fascicle enters the tissue and probes until it finds a capillary or vessel. Upon positioning of the fascicle, the bed bug becomes rapidly engorged with blood (Usinger 1966). Usually bed bugs feed when the host is asleep. They can take large blood meals due to the ability of their membranous abdomen to expand. It takes approximately ten minutes for bed bugs to take in two to five times their own body weight in blood. After engorgement the bed bug rapidly leaves the host to avoid detection (Usinger 1966). After leaving the host, the bed bug seeks out a harborage, like a crack or crevice,

in which to digest the blood meal (Slater and Baranowski 1978). Harborage sites are often on rough surfaces that are located close to the host. Bed bug harborages typically contain large amounts of feces, exuvia, egg casing, and other bed bugs of all developmental stages (Robinson 2005).

Bed bug bites are typically not painful for humans; however, the proteins contained in the saliva may cause allergic reactions. People differ in terms of reaction to bed bug bites. Skin reactions can be immediate or delayed. An immediate reaction usually appears within 24 hours, while a delayed reaction may appear a few days after the bite has taken place (Harlan 2007). Some bites can produce dermatitis, and prolonged scratching can result in secondary infections. In severe cases, bite victims may develop a hyperergic bullous, which is a allergic skin reaction similar to a pruritic rash (Liebold et al. 2003).

The fact that bed bugs take blood from multiple human hosts makes them good candidates for human disease vectors. Bed bugs have been shown to carry as many as 28 human pathogens. However, it has never been proven that bed bugs can vector or even mechanically transmit human diseases (Usinger 1966, Silverman et al. 2001, Blow et al. 2001). The potential for bed bugs to transmit HIV or hepatitis B virus (HBV) has been well studied. Jupp et al. (1978) and Webb et al. (1989) demonstrated that the HIV and the HBV viruses do not replicate inside of *C. hemipterus* and *C. lectularius* and therefore, bed bugs are unable to successfully transmit these diseases.

Even though bed bugs do not transmit diseases, living with a bed bug infestation does produce other health effects. People can experience sleep deprivation, anxiety, and stress. Moreover, there is a social stigma associated with bed bugs. Most people associate bed bugs with poor hygiene and unsanitary conditions so they are embarrassed about having bed bugs. In many

cases, social and personal relationships are negatively affected by the presence of bed bug infestations (Schaefer and Panizzi 2000).

C. lectularius development

The bed bug egg develops through five immature stages before becoming an adult (Fig. 2.1). As nymphs, bed bugs require a blood meal to complete each molt. Each developmental stage lasts for at least 6-7 days. Nymphs take a blood meal approximately three to five days after eclosion. However, the hatching and molting processes are influenced by environmental factors such as temperature and humidity, which can speed up or prolong the developmental process (Johnson 1941, Robinson 2005). As adults, a blood meal is required for egg production (Mellanby 1939, Johnson 1941, Davis 1964). Adults take a blood meal every 3-7 days if the host is available (Usinger 1966). An adult female bed bug can lay approximately 14 eggs per week. Eggs hatch within seven to nine days. The developmental period from egg to adult is approximately five weeks if the bed bug is able to take a blood meal every week (Lehane 1991).

Influence of temperature and humidity on *C. lectularius* development

Many studies have documented the influence of temperature on the bed bugs' developmental time (Bacot 1914, Mellanby 1934, Johnson 1940 and Omori 1941). Environmental temperature is the most influential factor, other than availability of blood meals, in determining bed bug development time. The lowest temperature at which bed bug eggs will hatch has been recorded as 8ºC (Johnson 1941). Based on this information Johnson (1941) studied the different temperatures and humidities at which bed bugs can develop after hatching. Most of the experiments were controlled at 23ºC, which was the common temperature for a

house in England during the summer in the 1940's. However, after measuring the effects of different temperatures, Johnson (1941) concluded that bed bugs kept at 28ºC developed faster than bed bugs kept at other temperatures and produced the maximum number of eggs possible. Therefore, 28ºC was recorded as the optimal temperature for bed bug development. Johnson (1941) also found that a relative humidity between 75-90% is the most favorable for rapid bed bug development.

Bed bug development can vary with temperature. At a high temperatures (28º-32ºC and 75-80% RH), Johnson (1941) found that eggs were able to hatch within 5-12 days. Nymphs at the same temperature and humidity (28º-32ºC and 75-80% RH) molted every 6-14 days. Therefore, under these conditions (28-32ºC, 75-80% RH) the minimum time for bed bug development from egg to adult was found to be as little as 40 days. If the environmental conditions are not optimal (e.g., lower temperature or irregular host availability) the developmental time from egg to adult can take up to 15 months (Harlan 2007). A wide range of humidities appeared to have little effect on the development time of *C. lectularius* (Johnson 1941).

Like all insects, extreme temperatures are fatal for bed bugs. However, at low temperatures it is difficult to find an exact degree and time of exposure to cause 100% mortality. Johnson (1941) exposed a clutch of eggs to 17º-18ºC for 2 h and observed that 75% of the eggs were dead after the exposure. Hase (1917) found that it is necessary to expose adults to 7ºC for 24 hours or -17ºC for 2 hours to achieve 100% mortality. High temperatures above 40ºC are sufficient to kill bed bugs. However, the lethal temperature again, depends on exposure time (Mellanby 1934). Mellanby (1935) demonstrated that bed bugs exposed to 45ºC reaches 100% mortality within one hour.

Starvation in *C. lectularius*

Nymph and adult bed bugs can survive long periods of time without a blood meal. Different studies have been conducted in order to determine the length in time that a bed bug can survive without a blood meal. According to Bacot (1914), newly emerged nymphs can survive without a blood meal for 136 days. These newly emerged nymphs survived ~ 9 months with a regular feeding schedule (65º F). Kemper (1930) starved bed bug nymphs to determine for how long they would survive. Kemper (1930) reported that nymphs (from different developmental stages) could survive without a blood meal for approximately 84 days. Gunn (1933) recorded that a pair of adult bed bugs survived for a "long time" with only occasional blood meals. Supposedly, the male lived to be four years old and the female lived to be three years old. The pair was fed approximately 23 times during the 3-4 year period. After the 3 year period, the pair was allowed to mate and they produced a few fertile eggs (Gunn 1933). Omori (1941) studied starved *C. lectularius* and *C. hemipterus* individuals from all developmental stages (1^{st}-5^{th} and adults) during starvation at different environmental temperatures (10°C, 18°C, 27°C, and 37°C) and 70-75% RH. Omori (1941) reported that individuals kept at lower temperatures (10°C-18°C) lived longer periods of time during starvation than individuals kept at higher temperatures (27°C-37°C). It was also reported that individuals of *C. lectularius* lived as long as 295.1, 277.1, 86.7, and 37.2 days after the last blood meal at 10°C, 18°C, 27°C and 37°C respectively. However, Omori (1941) did not specify if the study was conducted on recently molted individuals. Johnson (1941) reported that adult bed bugs lived 562 days without a blood meal after molting from fifth instar (13ºC and 90% RH). Johnson (1941) also stated that a house unoccupied for a long period of time (not specifically stated) could still have a bed bug population. Johnson (1941) reported

that the age distribution of bed bug populations in empty homes would consist mostly of fifth instars and adult males.

C. lectularius resurgence

Although bed bugs were nearly eradicated in the U.S in the 1950s, bed bugs have made a resurgence 50 years later. Beginning in the 1990s, reports of bed bug infestations began to increase in the United States (Cooper 2006). Pest control companies started reporting bed bug infestations that they had never seen before (Harlan 2006). Many pest management professionals had never experienced a bed bug infestation in their professional lives (Harlan 2006, Cabrera and Heinsohn 2006, Potter 2006). By 2001, 18 states reported bed bug infestations. In April 2004, the NPMA (National Pest Management Association) received samples of bed bugs from 4 providences in Canada, 3 states in Mexico and 40 states in the United States. As of 2005, there had been a significant increase in the number of bed bug calls received by pest management companies in most regions of the U.S. (Gangloff-Kaufmann et al. 2006, Cooper 2006). Pest control companies in the United States went from receiving sporadic calls about bed bugs to receiving bed bug calls every day (Cooper 2006). Pest management professionals in the U.S. believe that bed bugs are the most difficult pest with which they have had to deal (Potter 2007).

The United States is not the only country suffering from a bed bug resurgence. Nations like Korea (In-Yong et al. 2008), Israel (Mumcuoglu 2008), Great Britain (Reinhardt et al. 2008), Australia (Doggett et al. 2004), and Canada (Shemanchuk 1990) are also experiencing a bed bug resurgence.

The causes of the bed bug resurgence in the U.S. are unknown. However, the resurgence can be attributed to a number of factors that include an increase in international travel (Boase

2001), immigration from other countries (Cooper 2006, Potter 2007), recreational travel (Kells 2006), misidentification of bed bugs (Cooper 2006), and resistance to insecticides used for bed bug control (Potter 2006).

C. lectularius insecticide resistance

Insecticide resistance results from repeated applications of increasing rates and amounts of the same insecticide over time (Mullins and Scott 1992). The most common types of physiological resistance to insecticides are reduced cuticular penetration, alteration of the target site or nerve cell membrane so the target is not affected by the insecticide, increased or decreased metabolic detoxification so the insecticide is detoxified before it reaches the target site, and sequestration where the insecticide is stored in the body where is not harmful (Soderlund and Bloomquist 1990, Mullins and Scott 1992). Resistance to insecticides has been documented in more than 500 arthropod species (Roush and Daly 1990, Karunaratne et al. 2007).

Many insecticides, such us organophosphates and carbamates are no longer registered for indoor use in the U.S. Therefore, pyrethroids are the dominant insecticide class used for controlling bed bugs (Potter 2006). However, pyrethroid resistance has been well documented in *C. lectularius* (Moore and Miller 2006, Romero et al. 2007, Kyong et al. 2008) and in *C. hemipterus* (Karunaratne et al. 2007).

The most well studied type of physiological resistance is rdr resistance that is caused by α target site mutation. Yoon et al. (2008) found one mutation in the voltage-sensitive sodium channel α- subunit gene in almost all bed bug populations resistant to deltamethrin. A second mutation was also found in many of those same populations. The mutation found by Yoon et al. (2008) corresponds to the position of an important aminoacid residue associated with pyrethroid

sensitivity. The author also suggested that bed bugs may regulate their sodium channels in a different way than other channels in order to block the insecticide activity.

Resistance and fitness trade-off in *C. lectularius*

There is a trade-off between insecticide resistance and bed bug fitness. Fitness cost can be described as "a decrease in residual reproductive value as a consequence of parental effort". Fitness can be measured in terms of fecundity, developmental time, and life-span (Wood and Bishop 1981). Genetic changes that confer insecticide resistance can disturb normal physiology and use energy to detoxify insecticides that might otherwise be used for reproduction (Carriere et al. 1994). Alleles for resistance are very uncommon in insect populations due to the fact that these alleles can have a negative impact on fitness, such as a decrease in fecundity and longevity (Uyenomaya 1986, Groeters et al. 1994). Decrease in fitness as a consequence of insecticide resistance has been well documented in Aphididae (Hollinsgsworth et al. 1997), Tortricidae (Carriere et al. 1994), *Aedes aegyptti* (Culicidae) (Kumar et al. 2009), Muscidae (Krafsur et al. 1993, Roush and Plapp 1982, Scott 1997), and *Blattella germanica* (Grayson 1954). Kumar et al. (2009) found that *Aedes aegyptii* populations resistant to deltamethrin exhibited a 73-88% reduction in the gonotrophic cycles and therefore a 36.7% decrease in egg production. Scott et al. (1997) found that individuals from pyrethroids resistant populations of horn flies produced significantly fewer eggs than individuals from susceptible populations, also individuals from the resistant populations survived shorter periods of time than individuals from the susceptible populations. Roy et al. (2010) reported a significant decrease in fecundity in strains of the tea mosquito bug that were resistant to insecticides, when compared with the susceptible strains. In

addition, individuals from resistant populations of the tea mosquito bug had a longer developmental time than individuals from susceptible populations.

Control methods for *C. lectularius*

Bed bug control methods can be traced back to 1690 in Europe where exterminators used "secret formulas" to kill bed bugs. Some organic and inorganic insecticides applied as powders or fumigants for bed bug control were also highly toxic to humans. Some examples of these insecticides are sodium fluoride, phosphorus, thallium sulphate, hydrogen cyanide (Cornwell 1976), arsenic, mercury and burning sulfur or cyanide gas (Schaefer and Panizzi 2000).

During World War II many synthetic insecticides were developed. DDT (dichlorodiphenyl trichloroethane) was a very successful contact insecticide against vectors of louse bourne typhus and mosquito bourne malaria. Starting in 1942, DDT was used indoors for killing urban pests. DDT was very effective in killing bed bugs because of its long residual activity. In addition, DDT was inexpensive and could be used without any professional training. Therefore, DDT became the most widely used insecticide between the 1940's and 1960's. Resistance to DDT was reported by the 1950's. As a result the National Pest Association started to recommend other insecticides for bed bug control. Malathion, lindane, chlordane and dichlorvos were some of the pesticides recommended as alternatives to DDT for bed bug control. After 1972, the use of DDT was discontinued due to its longevity in the environment and the perception of danger to human health (Potter 2006).

After World War II different types of pyrethroids were produced. Pyrethroids are synthetic insecticides that have similar insecticidal properties to the natural insecticide, pyrethrum. Pyrethrum is derived from *Chrysantemum* flowers. The first synthetic pyrethroid

developed in the U.S. was allethrin (Cornwell 1976). Pyrethroids have low mammalian toxicity and became popular for their ability to kill a wide variety of insects at low concentrations. A few years after the development of pyrethroids, pest control companies started demanding that pyrethroid product for indoor use against bed bugs (Cornwell 1976). Since the development of the first pyrethroids many of the pyrethroid products were combined with a synergist to kill bed bugs faster. However, none of the pyrethroids had the residual activity of DDT (Potter 2007).

Today, almost all insecticides labeled for bed bug control are pyrethroids. Examples of insecticides that contain pyrethroids as their active ingredient include: DeltaDust (0.05% deltamethrin), D-Force (0.06% deltamethrin), Suspend (0.06% deltamethrin), Demand CS (0.03% lambda-cyhalothrin), Dragnet (0.05% permethrin), Talstar One (0.02% bifenthrin), and Tempo Ultra SC (0.07% beta-cyfluthrin). Some of the insecticides contain pyrethroids plus a synergist like Bedlam® (D-phenothrin). Insecticides currently labeled for bed bug control that contain a different active ingredient include: Phantom (0.5% chlorfenapyr) and Gentrol (hydroprene gentrol aerosol 0.36%) (Pinto and Associates, Inc. 2010).

Some insecticides labeled for bed bug control are effective at killing bed bugs by direct contact. However, because of bed bugs cryptic behavior, a large portion of the infestation does not come into contact with the insecticide. The lack of residual activity in these insecticides is one of the main reasons for bed bug control failure. To date there is not an effective residual spray insecticide labeled for bed bug control (Harlan 2006).

Alternative control methods for *C. lectularius*

Effective bed bug control cannot be limited to chemical products. Non-chemical methods must also be employed. These methods are not just an option, but a mandatory procedure to

reduce and control the bed bug infestations (Kells 2006). Non-chemical control methods include vacuuming, laundering of clothing and bedding, thermal treatments, and the reduction of bed bug harborages (Harlan 2007).

Vacuuming removes both live and dead bed bugs. It also helps remove the dirt allowing the chemical control methods to have better activity on treated surfaces. It is important to use disposable bags in the vacuum so that they easily can be removed from the premises (Doggett 2010).

According to Naylor and Boase (2010) washing and drying clothes and bedding can kill all bed bug developmental stages. Washing items in a regular cycle at 60°C kills all life stages. However, 40°C is sufficient to kill immature stages and adults, but not the eggs.

Heat is capable of killing all developmental bed bug stages at temperatures over 45°C. It is important to raise the temperature at once and not gradually, so the bed bugs can not disperse due to the rise in temperature (Doggett 2010).

Steam can be applied using water based steamers or dry steam. However, dry steam units are preferable because they reduce the time for drying items (Kells 2006 and Doggett 2010). Steam treatment should be used on the mattress, cushions of chairs and sofas, and carpet edges. Moreover, it is important to use the steamer unit in a low rate flow, using wide steam heads or covering the steam head with cloth to avoid blowing the bed bugs away (Doggett 2010).

Cold can also be used as an alternative for bed bug control. At about -20°C for over two hours, all immature stages and adults will be killed. Naylor and Boase (2010) recommend holding items in a conventional household freezer more than 10 hours. However, most household freezer are not cold enough to prevent the recovery of some adult bed bugs.

Mattress covers are a good alternative to reduce bed bug harborage and the insects' dispersion. Mattress encasements can be used to contain bed bugs that are already infesting the mattresses and box springs or to prevent that those items from becoming infested (Doggett 2010).

The economical cost associated with bed bug infestations may vary, but is always high. Most pest control companies charge hundreds of dollars (average $500 USD) for a single treatment, and three treatments at two week intervals are the standard practice. This cost includes three hours of inspection on average, insecticide application, and in some cases costumer education. Because the cost of control is so high, home owners and managers try to solve the problem by themselves. They tend to use home remedies and chemicals not labeled for indoor use. This temporary solution is not just ineffective but also potentially dangerous for human health. In hotels and hospitals bed bug control becomes even more challenging. Because of the size of the facilities, inspection can take many days. However, even if a large effort is spent in controlling bed bugs, a total elimination of the infestation by a single visit is almost impossible (Potter 2007).

Successful pest control relies on having basic ecological and biological knowledge of the pest (Pimentel 1966). Because bed bug populations were almost eradicated from the U.S., there is a gap in biological information between populations from 1940s and modern populations. As pointed out by Reinhardt and Siva-Jothy (2007), a better understanding of natural bed bug ecology is urgently needed. One of the tools available to entomologists for summarizing basic information regarding the ecology and population dynamics of an insect, is the life table (Harcourt 1969, Bellows and Birley 1981).

Life tables

A basic tenet of population ecology is that in the absence of migration, population growth rate and size are determined by two processes, birth and death (Andrewartha and Birch 1982, Carey 1993). The relationship between these two processes, birth and death, is the basis of demography, the study of populations and the processes that shape them (Pressat et al. 1985, Carey 1993). An in-depth look at the demography reveals data characterizing four aspects of populations (Shryock et al. 1976) size, the number of organisms in the population; distribution, the arrangement of the population in time and/or space; structure, the distribution of the sex and age groupings within the population; and change, the growth or decline of the population. Carey (1993) refers to size, distribution, and structure as population statics, and change as population dynamics. Of the two main components of demography, (i.e., formal demography and population studies) formal demography is the component, which is concerned mainly with population change (Hauser and Duncan 1959, Carey 1993).

In formal demography, biological attributes of a specific population, such as births and deaths, are studied to understand the changes that occur within the population and the factors that influence these changes (Allee et al. 1949, Krebs 2001). A basic tool in formal demography is the *life table*, which provides a method for tabulating and summarizing the birth and death events of individuals within a population, and as such, offers a simple way to study population changes. In practice, the life table, which has also been referred to as a 'book of death' (Price 1997), summarizes the age-specific mortality of a group of individuals or cohort within a population (Krebs 2001).

Life tables were first developed by demographers in insurance companies to assess the life length of individuals in the human population (Morris and Miller 1954, Young and Young

1998, Krebs 2001). Life insurance rates are based on the life expectancy of a person based on several physiological characteristics (Price 1997) Therefore, depending on the age and/or sex of the individual, a life insurance company will offer different rates for coverage in the event of death (Krebs 2001). Life tables were later adapted to study large animal and insect populations (Allee et al. 1949). Raymond Pearl (1927) was the first to introduce life tables to the field of ecology. One of the main outcomes of Pearl's studies was the formulation of three types of survivorship curves that graphically describe potential or probability of mortality as a function of age within a population. The three survivorship curves are: Type I, which describes populations with low mortality in immature stages and a high loss of older individuals (e.g., human); Type II, describes a population with a constant mortality rate independent of age (e.g., hydra); and Type III, describes a population with high mortality early in life followed by a constant loss of individuals over time (e.g., oyster) (Deevey 1947, Price 1984, Krebs 2001). The survivorship curves have been used to compare the probability of morality among different populations, and to determine the life stage or stages that are most vulnerable to mortality factors.

Morris and Miller (1954) were pioneers in adapting life tables for studying insect populations. The purpose of their original study was to determine the causes of mortality in the spruce budworm, *Choristoneura fumiferana*. Using life tables, Morris and Miller (1954) were able to identify important mortality factors, and to quantify population changes for this species. The application of life tables to entomological quedtions include studies of insects in the orders Phthiraptera (Evans and Smith 1952), Hemiptera (Hansen et al. 1999, Russo et al. 2004, Babin et al. 2008), Homoptera (Metcalfe 1972, Iversen and Harding 2007), Blattaria (Aguilera et al. 1997), Lepidoptera (Nanthagopal and Uthamasamy 1989, Kuhar et al. 2002, Satpute et al. 2005, Legaspi and Legaspi 2007), Coleoptera (Harcourt 1971, Bellows and Birley 1981), Diptera

(Carey 1982, Gabre et al. 2005, Afrane et al. 2007), and Hymenoptera (Coates 1976, Pilkington and Hoddle 2007). Life tables have also been used to study mite populations (Hansen et al. 1999, Andango et al. 2006). Not surprisingly, the main purpose for developing the life tables listed above was to gain a better understanding of the basic biology and ecology of the target species and/or to identify through key factor analysis (Morris 1959, Varley and Gradwell, 1960, 1970, Podoler and Rogers 1975) the major mortality factors affecting the population dynamics of a particular species.

In general, two basic types of life table have been developed for insects: a cohort life table and a current life table (Carey 1993, Krebs 2001). A cohort (horizontal, longitudinal, age-specific, or generational) life table summarizes the mortality experiences of a particular cohort from birth through consecutive ages until there are no individuals left from the original cohort. A current (vertical, cross-sectional, stationary, or time-specific) life table follows a hypothetical or synthetic cohort throughout its lifetime. The synthetic cohort is subjected to the age-specific mortality factors experienced by a real population over a specified period. Both types of life tables, i.e., cohort and current, can either be complete or abridged. A complete life table is one in which the measurements of the cohort are taken each day of the individual's entire life. In an abridged life table, daily survival/mortality measurements are difficult to obtain so that the age interval is usually greater than one day, such as a complete life stage (e.g., egg period). Cohort and current life tables can further be classified as single decrement, where all forms of death are lumped into one, or multiple decrements in which death is categorized by cause (Carey 1993).

Although most studies using life tables focus only on recording death within the population (mortality or survivorship life tables), some studies also include a record of births. These data are used to develop a fertility life table. The study of the human louse, *Pediculus*

humanus by Evans and Smith (1952) is a good example of the development of an age-specific, cohort, complete, combined mortality and fertility life tables for a species.

Finally, life tables also can be experimental or ecological. Experimental life tables deal with individuals reared artificially under controlled laboratory conditions. Ecological life tables, on the other hand, deal with populations in their natural environments. Ecological life tables, therefore, are more difficult to develop due to the fact that individuals are exposed to numerous hazards for which the impact on mortality is more difficult to predict (Andrewartha and Birch 1954).

A life table will usually contain several columns, which represent functions that describe the mortality or birth event of the cohort in relation to the age of the organism (Price 1997). The columns (functions) are related such that if information is available for one of the columns the values in all other columns can be calculated (Krebs 1999). Table 2.1 provides a description of and the method for calculating the values in the typical columns of a complete, mortality and fertility life table.

To construct a complete, single decrement, cohort life table it is necessary to observe a number of individuals (a cohort) and record the age-specific mortality of these individuals, and births, if the purpose is also to develop a fertility life table. In many life table studies, the number of individuals in the cohort is converted (or scaled) to a life table radix that represents the starting number of births at the beginning of the life table, against which survivors in each age interval are compared. In human studies, for example, the typical radix is 100,000 while in insect population biology the radix is often 1 or 1,000 (Carey 1993). For example, suppose an entomologist planned to develop a life table for an insect by following a cohort of 150 eggs (n_0), the researcher can convert the 150 individuals into a radix of 1.0 as follows: $(150/150) \times 1.0$.

The population at subsequent ages would then be scaled to the radix of 1.0 such that if 10 of the original cohort of 150 eggs died between day 1 and day 2, the proportion of the original eggs alive on day 2 would be $(140/n_0)*1.0 = (140/150)*1.0 = 0.9333$. The radix, therefore, is the initial value of the the survival column (l_x) of the life table, i.e., l_0.

Analysis of life table data (growth rate, development, and survival)

Any living organism is expected to grow at a certain rate, live for a particular period of time, and produce a certain number of offspring (Andrewartha and Birch 1954). In ecological studies, however, it is necessary to define these activities with respect to the population and not the individual. As such, it is customary to refer to mean development, mean survival, and mean fecundity of individuals in the population. These mean values of the population are determined by the interaction of the environment and the innate biological qualities of the species, which has been referred to as the innate capacity for increase, r_m (Andrewartha and Birch 1954). As defined by Andrewartha and Birch (1954), the innate capacity for increase is the maximal rate of increase of the population existing in an optimal environment. Its value depends on the difference between the birth-rate and the survival or death rate, such that a population increases if birth rate exceeds the death rate, and declines if death rate exceeds birth rate.

Although estimation of the innate capacity for increase appears to be straightforward, problems can arise in calculating this parameter because the birth and death rates vary with the age of the individual and the state of environment (Andrewartha and Birch 1954). Lotka (1925) solved this problem by considering r_m to be the rate of increase under optimal environment conditions. Lotka (1925) noted that in less than ideal environmental conditions one can only

estimate what the study called the intrinsic rate of natural increase, *r* (Birch 1948), which is also referred to as the instantaneous rate of population increase (Price 1997).

The intrinsic rate of increase (*r*), then, is the maximal rate of increase reached by a population at any particular combination of environmental factors and biological characteristics such as fecundity, longevity, and mortality. In order to calculate *r* it is necessary to extend the mortality (survival) life table for the adult stage into a fertility life table (Leslie and Park 1949). The columns of the fertility life table are shown at the bottom of Table 2.1.

From the columns of the fertility life table one can estimate the mean generation time of the population as $T = \sum l_x m_x x / \sum l_x m_x = \sum l_x m_x x / R_o$. The intrinsic rate of increase of the population then can be estimated as $r = \log_e R_o / T$. The *r*-value obtained by this method often is an underestimate of the true *r*-value (Birch 1948). A more precise estimate of *r* can be obtained iteratively using the relationship $\sum e^{-rx} l_x m_x = 1.0$. Statistical uncertainty in the value of *r* in the form of a standard error and 95% confidence interval can be derived using the jackknife or bootstrap procedures described in Meyer et al. (1986).

Often, an additional parameter, λ (antilog e^r), is calculated, which represents the finite rate of increase of the population (Birch 1948, Andrewartha and Birch 1954). The difference between *r* and λ is best defined using the example provided in Andrewartha and Birch (1954) who considered a population that multiplied 10 times every 2 weeks. The intrinsic rate of increase of such a population can be calculated using the equation for Malthusian growth,

$$N_t = N_0 e^{rt} \tag{2.1}$$

where N_t is the population size at time *t*, N_0 is the population size at time zero, and *r* is the intrinsic rate of increase. Rearranging equation (2.1) gives,

$$r = \frac{\log_e (N_t / N_o)}{t} \tag{2.2}$$

which for $t = 2$ weeks, $N_t = 10$, and $N_0 = 1$ results in,

$$r = \frac{\log_e (10/1)}{2} = 1.15 \tag{2.3}$$

The finite rate of increase (λ) of this population is then,

$$\lambda = e^r = \text{antilog}\, r = 3.16 \tag{2.4}$$

These results indicate that a population that multiplies 10 times every 2 weeks has an intrinsic rate of increase of 1.15 individuals/head/week and will multiply 3.16 times/week. As shown by Andrewartha and Birch (1954), after three weeks one individual will give rise to $(3.16)^3 \approx 32$ individuals. Such a population also would be expected to double in size in $D = (\ln 2/r) = (\ln 2/1.15) = 0.60$ weeks.

An example of the application of the above calculations for an urban pest, the human louse, *Pediculus humanus* L., can be found in Evans and Smith (1952). The intrinsic rate of increase, *r*, of *P. humanus* was estimated at 0.112 females/female/day with the finite rate of increase, λ = 1.118 females/female/day. Therefore, the louse population would be expected to increase 1.118 times/day and double in size every (ln 2/0.112) = 6.24 days. Evans and Smith (1952) also found that with an R_0-value of 30.93, the louse population would undergo a 31-fold increase in size each generation.

Life table data can also be used to estimate the duration of life stages and survival rates for the species under the conditions at which the study was conducted. Manly (1989) reviewed twenty one methods for analyzing the stage-frequency data of life tables to estimate stage duration and survival probabilities. Not included in the review are two methods, which are worth

mentioning. The method described by Carey (1993) is one that relies on a demographic approach for estimating the duration of each life stage from life table data. The other method is that of Pontius et al. (1989), which uses a nonparametric statistical approach. The two approaches are relatively simple and are comparable having been found to provide similar results (Carey 1993).

Life tables and pest management

Successful pest management relies on our understanding of the ecology of the pest population (Pimentel 1966). Because life tables provide static models that describe the dynamics of a population, they can be used to enhance our knowledge of pest biology, and as tools to improve our decision making in pest population management (Harcourt 1969, Bellows and Birley 1981, Iversen and Harding 2007). The use of life tables as a source of basic information on pest biology demonstrated by Evans and Smith (1952) who developed a life table for the human louse. Since 1952, life tables have been developed to gain insight into the biology and population dynamics of other insect species (e.g., Iversen and Harding 2007).

Pest managers can also use life tables to identify the vulnerable stages at which control techniques (e.g. insecticide) should be applied. If, for example, the life table for an insect shows that relatively high survivorship and long duration is to be expected during a particular life stage, this stage can be targeted for control in order to reduce population growth (Price 1997). Lagaspi and Legaspi (2007) used this argument to suggest that the egg stage of the cactus moth, *Cactoblastis cactorum* (Berg) should be the target for control. They argued that the exposed nature of eggs and the relatively long duration of the stage (based on life table data) made the egg stage the most vulnerable life stage for control.

Life tables also can be used to understand and compare life history characteristics among populations of the insects living under different conditions (Carey 1993). Arbogast (1975) and Russo et al. (2004), for example, used life tables to evaluate the potential of the predatory insect, *Xylocoris flavipes* (Reuter) under different temperatures and humidity in grain storage facilities. Life tables were also used by Andango et al. (2006) for a comparative analysis of the life history of the spider mite, *Tetranychus ludeni* on two hosts. Kuhar et al. (2002) developed life tables for the European corn borer to evaluate the effects of different inoculative releases of *Trichogramma ostriniae* on the pest population.

One use of life tables described by Harcourt (1969) and Southwood (1978) is as a source of information for the development of population models. According to Harcourt (1969), life tables help to "identify and evaluate the relative importance of the independent variables" causing mortality, which are then used to develop models for studying the behavior of the population under certain conditions. A good example of how life tables were combined with population modeling to study the population dynamics of the Mediterranean fruit fly is Carey (1982).

Population modeling

A "model" has been defined as a simplified representation or abstraction of a system or process, which is intended to enhance our ability to understand, predict, and also possibly influence the behavior of the system (Worner 1991, Goodenough and McKinion 1992, Peck 2000). This broad definition of a model is reflected by the many types and uses of this tool in the study of animal and plant populations. Geier and Clark (1976) classified models developed in ecology and pest management as descriptive or prescriptive. Peck (2000) on the other hand

categorized models as statistical (regression), process or descriptive, analytical, and simulation (computer). Statistical models are predictive and provide probabilistic interpretation of data (e.g., Kamminga et al. 2009). Descriptive models share many of the qualities of statistical models such as predictive ability, but also incorporate biological information (e.g., Legaspi et al. 1998). Analytical models the system can be represented in mathematical form as an equation or set of equations (e.g., Brewster and Allen 1997). Simulation models are those that rely on the use of computer programs to describe biological processes (e.g., Brewster et al. 1997). Descriptive, analytical, and simulation models also can be classified as discrete or continuous (in time and/or space), deterministic or stochastic, individual-based or population-based, and single or multiple species.

More often than not the models involve a combination of different types of modeling. For example, the model developed by Crouse et al. (1987) contains features of descriptive, analytical, discrete-time, deterministic, and single-species models. The model by Brewster and Allen (1991) is an example of a descriptive, analytical, simulation, continuous-time, deterministic, and multiple-species modeling for white flies.

The ability to combine the different types of modeling allows for models to be used for many types of ecological research and applied entomology. Pielou (1981) outlined four main uses of ecological models: to explain population dynamics, for forecasting future populations, to generate testable hypotheses, and to serve as standards for comparison with real systems. Worner (1991) reiterated the uses of models and also emphasized, as did Grier and Clark (1976) that models provide us with a structure with which to synthesize the information and data gathered from complex systems. Worner (1991) summed up the utility of modeling in applied entomology as follows: “when used for prediction in applied entomology, they [models] help us

put bounds on our uncertainty by allowing us to evaluate possible effects of different management practices and make more reasonable decisions than may be possible without models." Indeed, the ability of models to abstract and synthesize information for complex systems is one of the reasons why simulation modeling became the principal methodology used in early IPM research (Coulson and Saunders 1987).

With all of the different types of models and modeling frameworks, questions arise about whether to consider modeling, and which is the "best" modeling framework for the task at hand. Unfortunately, there are no simple answers to these questions. Nonetheless, many feel that modeling should only be undertaken given the right time (data and technical competence are available), right place (wide-ranging scope for the model), and right system (one that can be adequately delineated) (Geier and Clark 1976). In simple terms, modeling should only be undertaken if required to satisfy the objectives of the study, if there is a good supply of relevant data, and sufficient resources (time and money) are available to complete the exercise (Geier and Clark 1976, Worner 1991).

Population projection matrix models

The early models developed for use in IPM were mostly simulation models (Coulson and Saunders 1987, Bommarco 2001). However, because of the complexity of biological systems and mathematical intractability of modeling these systems, the amount of resources needed to develop these models, the unrealistic expectations of predictability, and the potential failure of the models (Pielou 1981, Worner 1991, Yu et al. 1992), pest managers were forced to turn to simpler modeling approaches. One such approach has involved the use of population projection-matrix models (Shea and Kelly 1998, Bommarco 2001). Projections matrix models can incorporate in simple fashion age- or stage-specific biological information on development,

mortality, and fertility of individuals in the population along with information regarding biotic (e.g., natural enemies) and abiotic (e.g. temperature) factors that might affect the population (Caswell 2001). These models can be analyzed analytically and/or used as a simulation model for projecting the population through time. As such, projection matrix models represent a middle ground in population modeling approaches between the predictability of statistical (regression) models and the complexity of simulation models (Yu et al. 1992).

Bernadelli (1941) and Lewis (1942) presented the first mathematical model of population growth that incorporated age structure. This matrix model, which was subsequently extensively developed by Leslie (1945, 1948), had the general form:

$$\mathbf{n}(t+1) = \mathbf{A}\mathbf{n}(t) \tag{2.5}$$

where $\mathbf{n}(t)$ and $\mathbf{n}(t+1)$ are vectors of population abundances at time t and $t+1$, respectively, and $\mathbf{A}$ is the population projection matrix. If we assume a hypothetical population with four age classes (C1, C2, C3, C4), with only the last age-class, C4, able to reproduce and provide individuals to the youngest age class, C1, equation (2.5) may be represented in matrix form as,

$$\begin{bmatrix} C1 \\ C2 \\ C3 \\ C4 \end{bmatrix}_{t+1} = \begin{bmatrix} 0 & 0 & 0 & F_4 \\ P_1 & 0 & 0 & 0 \\ 0 & P_2 & 0 & 0 \\ 0 & 0 & P3 & 0 \end{bmatrix} \begin{bmatrix} C1 \\ C2 \\ C3 \\ C4 \end{bmatrix}_t \tag{2.6}$$

where P_1, P_2, and P_3 are the survival probabilities for individuals in age-classes C1,C2, and C3, respectively, and F_4 is the fertility of individuals in age-class, C4. The projection matrix, $\mathbf{A}$ for this type of age-structured model is known as the Leslie age-class projection matrix (Caswell 2001).

Because it is often difficult to determine the age of individuals in populations for some species, such as insects, Lefkovitch (1965) modified the Leslie age-class matrix to account for stages or size differences rather than age. Therefore, if our hypothetical population was that of an insect having four life stages, egg (E), larva (L) pupa (Pp) and adult (Ad) instead of four age classes, the model in equation (2.6) becomes,

$$\begin{bmatrix} E \\ L \\ Pp \\ Ad \end{bmatrix}_{t+1} = \begin{bmatrix} P_1 & 0 & 0 & F_4 \\ G_1 & P_2 & 0 & 0 \\ 0 & G_2 & P_3 & 0 \\ 0 & 0 & G_3 & P_4 \end{bmatrix} \begin{bmatrix} E \\ L \\ Pp \\ Ad \end{bmatrix}_t \tag{2.7}$$

G_1, G_2, and G_3 represent the probabilities for individuals in the egg, larval, and pupal stages, respectively, surviving (S_i) and developing (D_i) to the next stage, i.e., $G_i = S_i D_i$. P_1, P_2, P_3, and P_4 represents the probability of individuals in the egg, larval, pupal, and adult stages, respectively, surviving and remaining to the same stage, i.e., $P_i = S_i(1 - D_i)$. The projection matrix, **A** in equation (2.7) is often referred to as a Lefkovitch projection matrix (Caswell 2001).

As a first step in developing the projection model in equations (2.6) or (2.7), Caswell (2001) suggested developing a life cycle or Coates graph of the projection matrix. If we considered the Lefkovitch projection matrix, **A**, in equation (2.7), the life cycle graph would be as shown in Fig, 2.2. The next step would be to develop a system of equations that represents the transitions between stages in the life cycle graph:

$$\begin{aligned} E_{t+1} &= P_1 E_t + F_4 Ad_t \\ L_{t+1} &= G_1 E_t + P_2 L_t \\ Pp_{t+1} &= G_2 L_t + P_3 Pp_t \\ Ad_{t+1} &= G_3 Pp_t + P_4 Ad_t \end{aligned} \tag{2.8}$$

The system of equations in equation (2.8) can then be rewritten in matrix form as shown in equation (2.7).

Data for parameterizing the transitions probabilities, G_i and P_i, can be obtained directly from field studies and/or life tables developed for the species (e.g., Carey 1982, Crouse et al. 1987, Shea and Kelly 1998, Bommarco 2001, Choi and Ryoo 2003). Once parameterized the projection matrix, $\boldsymbol{A}$, can be analyzed directly to derive the set of eigenvalues, λ_i, where i is equal to the size of the projection matrix. Therefore, for the projection matrix in equation (2.7), $i = 4$ so there would be four eigenvalues. The dominant (maximum) eigenvalue, often denoted λ_m (or λ_1 in some cases) represents the maximal growth rate of the population and is equal to e^r, where r is the intrinsic rate of increase of the population in the equation,

$$N_t = N_0 e^{rt} \tag{2.9}$$

When λ_m is 1, $e^r = 0$ and population size is considered to be stable. Analysis of the projection matrix, $\boldsymbol{A}$, also generates a right eigenvector, $\boldsymbol{w}_m$, such that

$$\boldsymbol{A}\boldsymbol{w}_m = \lambda_m \boldsymbol{w}_m \tag{2.10}$$

$\boldsymbol{w}_m$ represents the stable stage structure (stable age distribution) of the population, the proportion of individuals in each age or stage class to which the population would converge in a constant environment (Crouse et al. 1987, Caswell 2001). Also generated is the corresponding left eigenvector, $\boldsymbol{v}$`, calculated by,

$$\boldsymbol{v}`\boldsymbol{A} = \lambda_m \boldsymbol{v}` \tag{2.11}$$

which represents the reproductive values or the average contribution that each of the life stages makes to future generation size (Crouse et al. 1987, Caswell 1997, 2001). In general, the population projection matrix model can be analyzed several ways depending on the questions

one wishes to answer. Caswell (1996) categorized the main types of the analyses performed on the matrix population models as transient analyses, asymptotic analyses, and perturbations analyses.

Transient analyses and asymptotic analyses are closely related. Transient analyses focus on the short-term population dynamics using numerical projection to show what might happen to the population given specific initial conditions. Asymptotic analyses on the other hand focus on the long-term population dynamics. The derivation of the asymptotic growth rate and measures of population structure (i.e., stable stage distribution and reproductive values) described above are main components of asymptotic analyses. One of the main components of transient analyses is the determination of time to or rate of convergence of the population to a stable population structure (Caswell 1997, 2001). Bommarco (2001), for example, determined the time to convergence to stable stage distributions for several arthropod pests by starting with an initial population vector of one individual in the adult stage and zero individuals in the other life stages, and projecting each population into the future with their projection matrix. The time it took the projected population vector to match the stable stage distribution was then measured.

A more general approach was described by Caswell (2001) who noted that the rate of convergence of the population to a stable stage distribution can be determined by the relationship between the eigenvalue with the second largest magnitude, λ_2 and the dominant eigenvalue, λ_1. Caswell (2001) found that the larger λ_1 was relative to λ_2 the more rapid was the convergence to the stable age distribution. This relationship was defined by the damping ratio, ρ, as,

$$\rho = \frac{\lambda_1}{|\lambda_2|} \tag{2.12}$$

Convergence to a stable stage structure is considered to be approximately equal to log ρ. Unfortunately, the factors that influence the damping ratio in stage-structured models have not been well studied (Caswell 2001).

Caswell (2001) also described methods for measuring the distance between two stage distributions, either for two individual species or between an observed and predicted distributions. One such method is Keyfitz Δ (Keyfitz 1968), which essentially measures the distance between two probability distributions. Keyfitz Δ ranges from 0–1 with a value of 0 indicating that the two distributions are identical (Caswell 2001). Finally, two stable stage distributions can be compared statistically to test the null hypothesis of no difference between the distributions (Brault and Caswell 1993).

Perturbation analyses are used to examine the effects of changes in vital rate parameter values or initial conditions on the dynamics of the population (Caswell 1997, 2001). According to Caswell (1996), perturbation analyses attempts to answer certain specific questions. Questions such as "what are the effects of potential changes in the vital rates" as might occur with strategies that are designed to control a pest population? "Where efforts to improve estimates of the vital rates should be focused?" since better estimates of population growth would be obtained from better estimates of the vital rate to which growth rate is most sensitive. Also, "how do vital rate differences contribute to observed differences in growth rates" among two or more populations of the species?

The first two questions above are topics for perceptive perturbation analyses, while the third question is best answered using retrospective perturbation analyses. Perspective analyses, examines the effects of potential future changes, by asking the question what would happen to some dependent variable (such as population growth rate) if vital rates were to change (Caswell

1997, 2001, Horvitz et al. 1996). Sensitivity and elasticity analyses are both forms of perspective analyses, but provide answers to different questions (Caswell 1969). Sensitivity analysis measures the rate of change on a linear scale, while elasticity analysis measures the proportional change. Because elasticities sum to 1.0 (deKroon et al. 1986) comparisons of the relative contributions of the vital rates in the projection matrix (i.e., F_A, P_i, and G_i) to population growth rate, λ_m becomes easy. Because of this, elasticity analysis is often preferred over the sensitivity analysis.

Retrospective analysis involves examining the effects of vital rates on population changes that have already occurred. Whereas, perspective analysis attempts to predict how changes in vital rates might affect population growth rate, retrospective analysis is interested in explaining which vital rates were actually responsible for the observed changes in growth rate (Caswell 1997, Horvitz et al. 1996). One of the tools of retrospective analysis is the Life Table Response Experiments (LTRE), which bears a lot of similarity to traditional manipulative and observational experiments (Brault and Caswell 1993, Horvitz et al. 1996, Caswell 2001) such as having specific designs (e.g., factorial design or random design) for examining treatment effects (e.g., pesticide rates) on the response variable (population growth rate).

To date very little information is available on the ecology and life history of bed bug populations in the U.S, and on management tactics that are specific to different infestations. We know little about the fecundity, survivorship, and dynamics of modern populations of bed bugs. The different conditions under which modern bed bug populations have evolved could have had a significant effect on their development and fecundity. Developing life tables and mathematical models for modern bed bug populations will help quantify changes in the development, survivorship, and fecundity of populations under specific conditions. In addition, life tables They

will allow for comparing life history parameters of pesticide resistance and susceptible populations of bed bugs.

Table 2.1. Columns of a typical insect survivorship and fertility life table

Column (Function)	Description	Calculation
x	Age or interval in appropriate units (e.g., day)	
n_x	Number of individuals alive at age or interval, x. n_0 = number in the original cohort at start of life table	
l_x	Proportion of individuals alive at age x. The first value of this column, l_0, is the life table radix	$\frac{n_x}{n_0}$
p_x	Proportion of individuals surviving from x to x+1	$\frac{l_{x+1}}{l_x}$
q_x	Proportion of individuals dying from x to x+1	$\frac{d_x}{l_x} = \frac{l_x - l_{x+1}}{l_x} = 1 - p_x$
d_x	Rate of mortality. Proportion dying in interval, x to x+1	$l_x - l_{x+1}$
L_x	Number of days lived by the average individual in the cohort from age x to x+1	$(l_x - d_x) + 0.5d_x$ $= \frac{l_x + l_{x+1}}{2}$
T_x	Total number of days to be lived by the average individual within the cohort beyond age x	$\sum_{y=x}^{\omega} L_y$
$\boldsymbol{e_x}$	Expectation of life. Mean length of life remaining to each individual alive at the beginning of the age interval, x	$\frac{T_x}{l_x}$

Column (Function)	Description	Calculation
Columns in the Fertility Life Table		
m_x	Expected daughters – number of daughters a female is expected to leave at each age interval in x. $\sum m_x$ = Gross Reproductive Rate, the expected number of daughters produced by a female who lives through all of the age intervals.	
$l_x m_x$	Reproductive Expectation of a female at age x. $\sum l_x m_x$ = Net Replacement Rate (R_0), the total number of daughters that replace the average female in a generation or the rate of multiplication of the population in one generation (Birch 1948). $R_0 = 1$ for a stable population.	

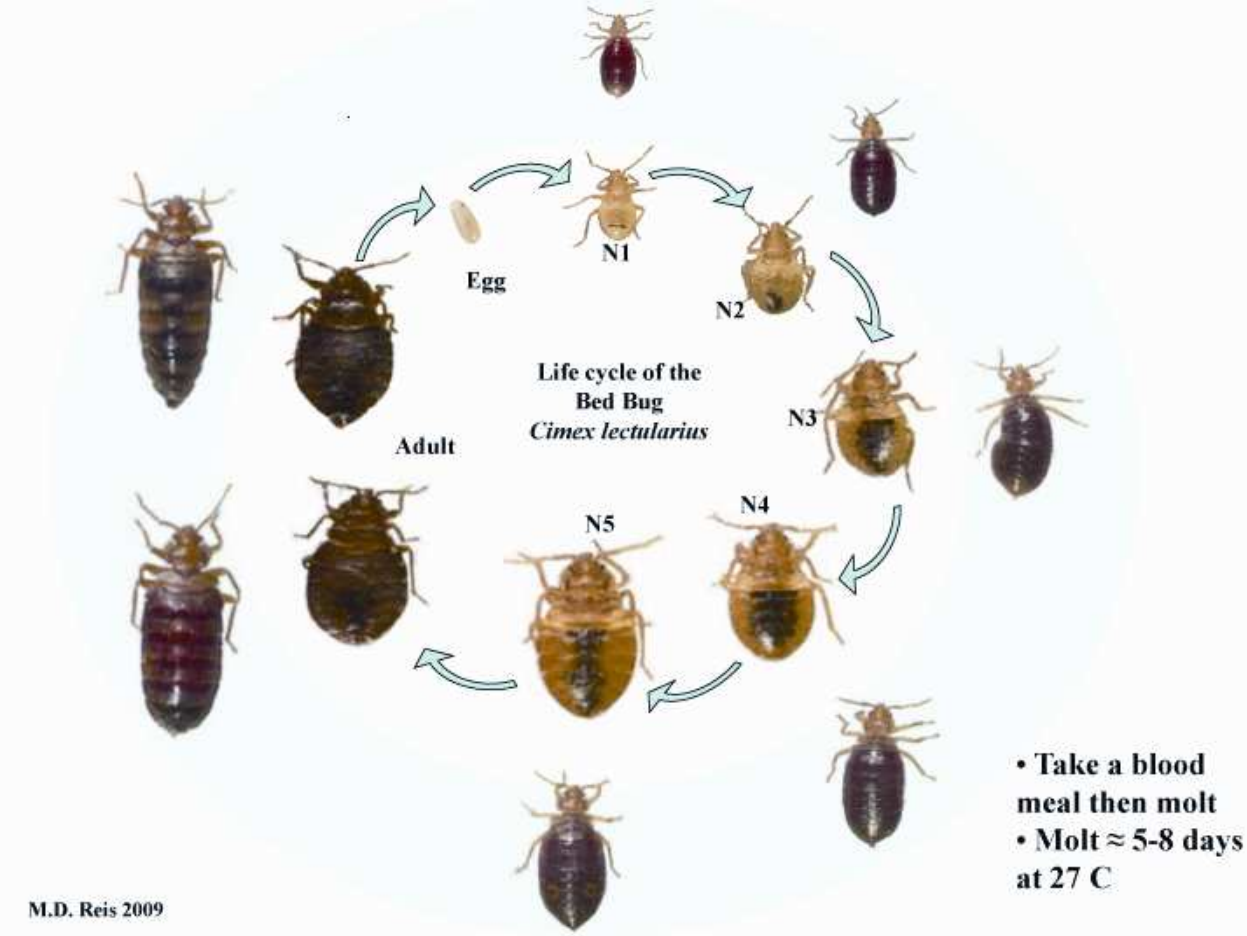

Figure 2.1. Life cycle graph of the seven stages of the common bed bug, *Cimex lectularius*. Egg, nymphal stage 1-5 and adults. Immature stages and adults bed bug are shown in both the fed and unfed state (Used under fair use).

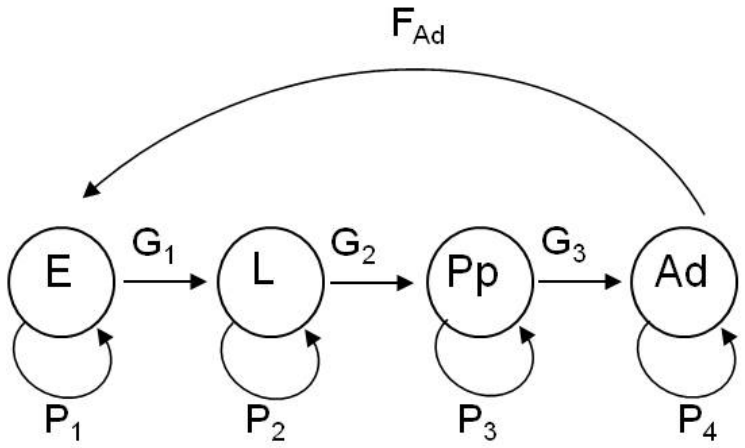

Figure 2.2. Coates life cycle graph of four stages of a hypothetical organism. E = egg stage, L = larval stage, Pp = pupal stage and Ad is the adult stage; $G_i = S_i D_i$, is the probability of individuals in the ith stage surviving (S_i) and developing (D_i) to the next stage; $P_i = S_i(1 - D_i)$, is the probability of individuals in the ith stage surviving (S_i) and not developing $(1 - D_i)$ to the next stage; F_{Ad} is female fecundity.

Chapter 3: *C. lectularius* L. Egg Production and the Cost of Traumatic Insemination

Introduction

Within the family Cimicidae there are three species, which are human ectoparasites: *Cimex hemipterus, Leptocimex boueti* and *Cimex lectularius. C. lectularius* is a pest in the temperate latitudes and the species currently infesting the U.S. Although these species can also feed on other animals (birds, bats, etc) the preferred host is human. All of the immatures stages of *Cimex lectularius* must feed on blood in order to molt. The frequency of feeding directly affects the bed bug nymphs' developmental time. Taking partial blood meals can delay the process of molting to the next developmental stage. In absence of a blood meal, bed bug nymphs do not develop to the next instar. Adult male and female bed bugs also require blood meals to reproduce (Usinger 1966). The frequency of feeding by *C. lectularius* directly affects the egg production in adults. For instance, the larger the blood meal the greater the number of eggs produced by the adult females (Usinger 1966). The host's blood seems to have an effect on the number of eggs laid by the female bed bugs after a blood meal. Johnson (1941) reported that female bed bugs fed on mice produced the greatest number of eggs than when they fed on other hosts. Usinger (1966) reported a significant difference in the number of eggs laid by females fed on rabbits than females fed on chickens, humans, or pigeons.

C. lectularius has a particular method of reproduction called "traumatic insemination" (Usinger 1966), which begins with the adult male piercing the abdominal cavity of the female. The male then uses his reproductive organ, the paramere, to inseminate the female directly into her abdominal cavity (Carayon 1966). During copulation the male paramere causes cuticular wounds in the female that leave permanent scars on her abdomen (Usinger 1966). Multiple traumatic inseminations can be costly for females, reducing her fitness in terms of life span and

reproduction (Siva-Jothy 2006). Even though extra-genital insemination is a rare phenomenon, it has been observed in many invertebrate taxa (Stutt and Siva-Jothy 2001).However, extra-genital insemination or "traumatic insemination" in bed bugs has not been well studied. The most comprehensive works are from Stutt and Siva-Jothy (2001), Reinhardt et al. (2003), Siva-Jothy and Stutt (2003), and Siva-Jothy (2006).

Stutt and Siva-Jothy (2001) studied seven different populations of bed bugs to quantify the cost of multiple matings. However, the levels of resistance in these bed bug populations was not assessed. Stutt and Siva-Jothy (2001) found that insemination once every four blood meals is enough to maintain reproductive success in a population of bed bugs. However, the rate of copulation in natural environments may be 20 times more than necessary to maintain maximum fertility. As a result of repeated "traumatic insemination" it was determined that female bed bugs had a 24% reduction in reproductive output.

Reinhardt et al. (2003) and Morrow and Arnqvist (2003) determined that the female sperm storage organ, the spermalege, can be a counteradaptation to traumatic insemination. Male bed bugs introduce their reproductive organ into the spermalege during the process of insemination. Male bed bugs can introduce microbes through their reproductive organ into the females body during copulation. Reinhardt et al. (2003) found that when needles infected with microbes where introduced into the female spermalege the number of bacteria was lower than in other abdominal locations where the infected needles where introduced. Morrow and Arnqvist (2003) also found that the female's spermalege reduce the cost of traumatic insemination in female bed bugs. Siva-Jothy (2006) found that the presence of microbes in the male's reproductive organ potentially decreased the female's fitness in terms of egg production and life-span. Infection activated the female's immune system which was energetically costly.

This study aims to quantify and compare female bed bug egg production for resistant populations over the course of multiple blood meals. We also quantify and compare egg production for female subject to single or repeated traumatic inseminations.

Materials and Methods

Bed bug rearing

Two field strains, Richmond (RR) and Nottingham Green (NR) were collected from apartments in Richmond, VA in September 2008 and July 2009, respectively. Another field strain, Epic Center (ER) was collected in June 2008 from a hotel room in Cincinnati, OH. The bed bug colonies were reared in plastic jars covered at one end with a cloth mesh. Two pieces of cardboard were placed inside of the jars so that bed bugs could crawl up the cardboard and stick their mouth-parts through the cloth mesh to feed on an artificial feeder. An artificial feeder using circulating hot water maintained a diet of chicken blood (sodium citrate as an anticoagulant) at 35.5º C. Bed bugs were fed once a week on chicken blood formulated with sodium citrate as an anti-coagulant. Between feedings, bed bugs were stored in an environmental chamber at temperatures between 26.1º C - 26.5º C, 68.9% RH and photoperiod of 12:12 h, light and darkness. These conditions were considered optimal for *C. lectularius* and closely approximate to the conditions at which Johnson (1941) evaluated bed bug populations for maximum fecundity and longevity. All bed bug colonies were maintained in the Dodson Urban Pest Management Laboratory at Virginia Tech, Blacksburg, VA.

Assessment of insecticide resistance status

The status of each bed bug strain, with regard to insecticide resistance was assessed prior to evaluations of fecundity. The strains evaluated were the Harlan laboratory strain (H), and the Epic Center (E), Nottingham Green (N), and British (B) field strains. The letter S was assigned to the strains susceptible to pyrethroids, and the letter R to the resistant ones. The bed bug populations were evaluated using the standard procedure (Moore and Miller 2006) of exposing

groups of bed bugs to a pesticide-treated surfaces and recording their mortality at regular time intervals. Groups of ten adult bed bugs (1:1 sex ratio) were fed to repletion 5 d before testing. Bed bugs were then transferred from rearing jars into closed Petri dishes (Fisher brand 35 x 10 mm). Hardboard panels (4 x 4 cm) were treated to the point runoff with a test insecticide formulated at the label rate. The insecticides tested were deltamethrin (0.06%; Suspend SC; Bayer Environmental Science, Montvale, NJ), and permethrin (0.05%; Dragnet SFR; FMC Corp, NJ). Control panels were treated with water only. The panels were allowed to dry, the Petri dishes were opened and inverted onto the treated surface, so that the bed bugs were confined inside the dish while in direct contact with the treated surface. Mortality was recorded at regular intervals and each pesticide bioassay for each bed bug strain was replicated five times. LT_{50} values were calculated for each strain exposed to each pyrethroid formulation. If the LT_{50} value calculated for a particular field strain was 10-fold greater than that calculated for the laboratory strain, the strain was considered to be resistant (Cochran 1993).

Evaluation of life long egg production potential (multiple matings)

Three pyrethroid-resistant field strains (RR, ER, and NR) were evaluated to determine their egg production potential. Twenty male and female 5^{th} instars were selected from each bed bug strain and were allowed to feed and then molt to adulthood. The process of feeding the 5th instars was conducted by inverting the mesh covered opening of the rearing jars against the arm of a human volunteer. Bed bug feeding was conducted as approved by the Virginia Tech Institutional Review Board (IRB 06-165). Once the bed bugs molted into adults, they were fed again on a human volunteer as described above. After feeding, pairs of adults (1 male: 1 female) were placed in individual Petri dishes (Fisher brand 60 x 15 mm) containing a single piece of

filter paper (Whatman 42.5 mm) and left to produce eggs. The filter paper in each Petri dish was replaced daily and the number of eggs on the paper was counted and recorded until egg laying ceased. A blood meal was offered on the second or third day after the cessation of oviposition as described by Johnson (1941). Egg laying was continuously recorded for 13 feedings/oviposition cycles or until the adult female died.

Comparison of fecundity: single versus multiple matings

Virgin female bed bugs from the RR field strain were collected on the day after adult eclosion. Females were then allocated at random to one of two experimental treatments. In the first treatment, 20 females were fed to repletation as described above, then allowed to copulate once with a virgin male before being isolated in a Petri dishes (Fisher brand 60 x 15 mm) containing a single filter paper (Whatman 42.5 mm). The mated females were fed every 7 d and the number of eggs produced was counted each week for five feeding/oviposition cycles. A second group of virgin females were fed and placed in a Petri dish with a fed virgin male. The pair was allowed to copulate repeatedly throughout the test period. The mating couples were fed every 7 d and the number of eggs produced each week for 5 feeding/oviposition cycles were counted and recorded. Only fertile eggs were counted in each treatment.

Statistical analysis

For the assessment of pyrethroid resistance, the chi-square and LT_{50} values calculated for each strain exposed to each pesticide were calculated using Probit analysis (Robertson et al. 2003). LT_{50} values were calculated for each strain and for each pesticide. Significant differences

between the treatments were determined by the failure of the 95% confident intervals (CI) to overlap.

When evaluating the life-long egg production potential, the overall mean number of eggs oviposited by females of the RR, ER, and NR strains was calculated. Repeated measures MANOVA was used to determine differences in egg production between bed bug strains. The egg production data for each strain were transformed ($\sqrt{y+0.5}$) and analyzed, using repeated measures Multivariate Analysis of Variance (MANOVA) (Ott and Longnecker 2001, Norman and Streiner 2008). Two separate analyses were performed using either feeding/oviposition cycle, or day after feeding, as the repeated measures factor.

When evaluating the single mating versus multiple matings the overall mean number of eggs oviposited by RR females was calculated. Significant differences in mean egg production between the single mating and multiple mating status groups were determined using repeated measures MANOVA. The egg production data for each strain were transformed ($\sqrt{y+0.5}$) and analyzed, using repeated measures Multivariate Analysis of Variance (MANOVA) (Ott and Longnecker 2001, Norman and Streiner 2008). Two separate analyses were performed using either feeding/oviposition cycle, or day after feeding, as the repeated measures factor.

Repeated measures MANOVA treated the response variable (number of eggs) at each observation point (feeding/oviposition cycle or day after feeding) as a different variable. MANOVA is often preferred over repeated measures ANOVA especially when the sampling intervals are not equally spaced (Norman and Streiner 2008). The repeated measures MANOVA consisted of analyses of two effects, a between-subject effect of the main factor (bed bug strain) and a within-subject effect that included time (feeding/oviposition cycle or day after feeding).

For the analysis of the within-subject effect, significant time factor × main factor interactions were examined to determine how the patterns of oviposition across each of the time factors differed with respect to each strain (Ott and Longnecker 2001). A sphericity χ^2 test, generated as part of within-subject analysis, was used to check the sphericity assumption (equal variances and correlations across time in the response variable) and appropriateness of using the unadjusted univariate *F*-test values to determine significance. If the sphericity test was not significant, the unadjusted values of the *F*-test were reported, otherwise the adjusted values, including adjusted degrees of freedom, were reported (Ott and Longnecker 2001, Norman and Streiner 2008). In all analyses where significant differences ($\alpha = 0.05$) were detected, multiple comparisons of the mean responses of the factor levels were carried out by orthogonal contrast (Ott and Longnecker 2001). All statistical analyses were carried out using JMP 8.0 (SAS Institute, 2005).

In addition to the above analyses, a predictive model

$$Y = aX \exp(-bX) \tag{3.1}$$

was fitted to the data on the mean number of eggs/female/day . In the model, *Y* is the predicted mean number of eggs/female/day, *X* is the feeding/oviposition cycle number, and *a* and *b* are constants (Hansen et al. 1999). The parameters of the model were estimated using nonlinear least-squares in TableCurve 2D 5.01 (SYSTAT Software Inc., Richmond, CA).

Results

Assessment of insecticide resistance status

The results of the LT_{50} analysis indicated that both of the pyrethroid insecticides killed the Harlan laboratory strain (HS) and the British strain bed bugs (BS) relatively quickly (range 1.3 - 3.3 hours) (Table 3.1). Both deltamethrin and permethrin produced LT_{50} values of less than 4 hours for both strains. The Harlan strain was significantly more susceptible to deltamethrin than the British strain.

Field strains were significantly less susceptible to the pyrethroid formulations than Harlan laboratory strain (HS) and British field strain (BS). The LT_{50} for the Richmond (RR) field strain bed bugs exposed to deltamethrin was 13 d and 3 h. This value was 390 times greater than the LT_{50} values calculated for the laboratory strain (0.8 h). The LT_{50} for RR exposed to permethrin was > 18 d and could not be calculated due to mortality in the controls occurring prior to deltamethrin treated insects reaching 80% mortality. This value was >300 times than the LT_{50} values calculated for the laboratory strain (1.5 h) (Table 3.1).

The LT_{50} for the Nottingham green (NR) field strain bed bugs exposed to deltamethrin and permethrin was > 21 d but could not be calculated. Therefore, the (NR) strain was determined to be at least 500 times less susceptible to deltamethrin and permethrin than the Harlan laboratory strain (0.9 h; 3.9 h) (Table 3.1).

The LT_{50} for the Epic center (ER) field strain bed bugs exposed to deltamethrin was > 16 d but could not be calculated. Therefore, the (ER) strain was determine to be al least 340 times less susceptible to deltamethrin and permethrin than the Harlan laboratory strain (1.9 h; 1.1 h) (Table 3.1).

Evaluation of lifelong egg production potential

Fig. 3.1 shows the average number of eggs laid by females of the Richmond (RR), Nottingham Green (NR), and Epic Center (ER) pyrethroid-resistant strains during 13 feeding/oviposition cycles. At the end of the experiment almost all females from the field strains were dead. At the end of the 13-wk study, 20 out of 20 females from RR strain were dead. 13 out of 20 females from NR strain were dead at the end of the study. For the ER strain 15 of the 20 females has died at the end of the experiment. The overall mean (± 95% CL) number of eggs oviposited per female during all of the 13 feeding/oviposition cycle for the RR, NR, and ER strains were 131.9 (± 2.57 SE), 138.5 (± 2.43 SE), and 155.6 (± 2.41 SE), respectively. The mean number of eggs produced per female per week for the RR, NR, and ER strains were 8.75 (± 0.51 SE), 9.27 (± 0.45 SE), and 8.86 (± 0.48 SE), respectively. The overlapping confidence intervals indicated that there were no significant differences among the mean number of eggs oviposited by the three strains of bed bugs over the course of the study.

The between-subject analysis of the repeated measures MANOVA indicated that there were no significant differences among the three bed bug strains in the mean number of eggs produced/female/day over the course of the 13 feeding/oviposition cycles ($F = 1.86$; df = 2, 597; $P > 0.05$). However, significant differences ($F = 7.32$; df = 24, 7140; $P < 0.0001$) were observed in the 13 week pattern among the three strains. This difference in the oviposition trends (decreased production overtime) was even more obvious when fitting the model equation to the egg data (Fig. 3.2). The model indicates a peak in the egg production for the three bed bug strains between the 3rd and 4th feeding cycles. Then a rapid decline occured in oviposition between the 6th and 8th feeding cycles (Fig. 3.2).

The average peak egg production for the field strains occurred during the fourth feeding/oviposition cycle. On average females from the field strains produced 0.74 eggs per day during the 13 feeding/oviposition cycles. The egg production patterns for the three strains between feedings are presented in Fig. 3.3. Although there were no significant differences among bed bug strains in the mean number of eggs produced/female/feeding ($F = 1.46$; df = 2, 773; $P > 0.05$), the patterns of daily oviposition between feedings were significantly different between the three strains ($F = 41.65$; df = 18, 6957; $P < 0.0001$). The (ER) begin egg production ~ two days later than the other two strains (Fig 3.3). On average, the oviposition cycle between feedings was 10 d. Females from each strain started producing eggs on ~ 3 days after feeding, with maximum egg production occurring between the 5th day and 8th day.

Comparison of fecundity: single versus multiple matings

Females isolated from males after a single mating produced on average 66.7 ± 0.84 SE eggs per female over the five feeding/oviposition cycles. Females that were exposed to multiple copulations produced on average 48.6 ± 0.69 eggs per female over the five feeding/oviposition cycles. Isolated females produced 27 % more eggs than the females exposed to multiple matings. Females that were exposed to constant mating during the five feeding/oviposition cycle experiments produced a significantly fewer number of eggs than the females that were isolated from male after a single mating ($P = 0.0019$) (Figure 3.4). However, the egg production pattern between isolated females and females exposed to constant mating was not significantly different ($P = 0.1201$).

Discussion

In this study, the LT_{50} values calculated for the three bed bug field strains (RR, ER, and NR) exposed to pyrethroid insecticides were significantly greater than the susceptible laboratory strain (HS). If resistant ratios could have been calculated for each strain exposed to each pyrethroid they would have been (Cochran 1993) > 300. Identifying these strains as resistant is important when assessing life history parameters because several studies have demonstrated that resistant insect populations typically produce fewer offspring, have a longer developmental time and higher mortality than susceptible populations. Differences in reproductive output between susceptible and resistant populations have been studied in the family Miridae (Roy et al. 2010), Muscidae (Roush and Plapp 1982, Scott et al. 1997), Aphididae (Hollingsworth et al. 1997), and in individuals from German cockroaches (Grayson 1954), and *Aedes aegypti* (Kumar et al. 2009).

In this study, bed bug females from three pyrethroid-resistant field strains (Richmond – RR, Epic Center – ER, and Nottingham Green –NR) produced eggs for a period of 10 d after a blood meal. These results were similar to those reported by Johnson (1940) (8–10 d egg producetion after feeding) for the same species. We observed that female bed bugs started producing eggs on the 3rd d after the blood meal. Johnson (1940) also found that at 27°C the first eggs were laid 3 days after the blood meal. How and Lee (2010) also found that egg production began 2–5 d after a blood meal in *Cimex hemipterus*. In our study, maximum egg production occurred during the first 4 feeding/oviposition cycles with a decline beginning after 6 weeks. Johnson (1940) also determined that peak egg production for *C. lectularius* took place during the fourth oviposition/feeding period. How and Lee (2010) reported that egg production in *C. hemipterus* peaked at the second and third oviposition cycle.

On average females of the three pyrethroid-resistant strains (ER, NR, and RR) produced 156, 139, and 132 eggs during the 13th feeding/oviposition cycle respectively. The number of eggs produced by females reported prior to 1940 (probably at room temperature 18°C - 20°C) were incredibly high compared to those reported in our study. Titschack (1930) gives 541 as the highest number of eggs produced by a single female in her lifetime. Female bed bugs in our experiment produced on average 0.74 eggs/d. Hase (1917) gives 12 as the maximum number of eggs laid in a day by a single female bed bug. Differences in egg production may be positively correlated with amount of blood meal and female's size (Johnson 1941). In addition, blood from different host can have an effect in the eggs produced by female bed bugs. Johnson (1941) found that female bed bugs fed on mouse laid the greatest number of eggs compared with females fed on fowl and human blood.

We found no significant differences in the average egg production between the three bed bug field strains (ER, NR, and RR). How and Lee (2010) also found no significant differences in the number of eggs produced by six field strains of tropical bed bugs. No significant differences suggest that field populations of *Cimex* do not necessarily differ in terms of fertility. However, further studies are needed to assess a greater number of field populations.

In this study, the egg production decreased drastically after the 12th and 13th feeding/oviposition cycles. Also the majority of the female bed bugs were dead at the end of the 13 feeding/oviposition cycle. Johnson (1940) stated that female bed bugs become less fertile with age. However, this study also suggests that repeated copulation also reduces the number of eggs produced over time. This is not surprising in light of the energetic costs of traumatic insemination (Stutt and Siva-Jothy 2001).

In our study, female bed bugs exposed to a single mating produced a significantly greater number of eggs than those exposed to multiple matings. The differences in egg production may be attributed to the "traumatic insemination". Repeated copulations can result in cuticular wounds in the female's abdomen due to the forced entry of the male's reproductive organ (Usinger 1966). The healing of these wounds can be energetically costly for females (Chapman 1998). Interestingly, Stutt and Siva-Jothy (2001) stated that female bed bugs were often subjected to traumatic insemination 20 times more than was necessary to maintain maximum fertility. Stutt and Siva-Jothy (2001) also reported that regular copulation decreased female longevity without egg production compensation. Another potential cost of traumatic insemination is associated with the immunological response in female bed bugs as her body battles potential infections that can result from multiple copulations (Siva-Jothy 2006 and Lonchmiller and Deerenberg 2000). Reinhardt et al. (2003) found that female bed bugs pierced in the abdomen with needles contaminated with bacteria had an 89.7% reduction in lifespan and therefore in reproductive output.

Our study suggests that each female bed bug from three pyrethroid resistant populations can produce an average of ~140 eggs during a period of 13 feeding/oviposition cycles. After which, her death is likely to occur. There were no significant differences in the number of eggs produced by females from different strains ($P > 0.05$). However, the daily pattern of oviposition was significantly different among the three bed bug strains during 13 feeding/oviposition cycles. Our results also found that when a female bed bug was isolated from males after a single mating, she produced 27% more eggs than a female expose to multiple matings. Because a single mated female bed bug only needs access to regular blood, we can suggest that a single mated female bed bug is capable of initiating an infestation. Access to a host is all that will be necessary for a

single-mated female to start producing eggs, and establish a new population.

Table 3.1. Time to mortality of adult bed bugs (50% male: 50% female) confined on hardboard panels treated with residual insecticide. Samples run same day for comparison.

Bed Bug Strain	Treatment[a]	LT_{50} (h)	95% CLs	Resistant ratios	Test Date
Harlan (HS)	Suspend SC	0.8	0.72–0.94		February 2009
	Dragnet	1.5	1.23–1.78		
Richmond (RR)	Suspend SC	320.2	304.4–338.2	390.5	February 2009
	Dragnet	>432	---	291.7	
Harlan (HS)	Suspend SC	0.5	0.47–0.54		July 2010
	Dragnet	3.9	3.67–4.19		
British (BS)	Suspend SC	1.3	1.30–1.37	2.6	July 2010
	Dragnet	3.3	2.65–3.89	0.8	
Harlan (HS)	Suspend SC	1.0	0.90–1.01		September 2010
	Dragnet	3.9	3.67–4.19		
Nottigham green (NR)	Suspend SC	>504	---	>500	September 2010
	Dragnet	>504	---	>500	
Harlan (HS)	Suspend SC	1.1	1.06–1.19		August 2009
	Dragnet	1.9	1.57–2.13		
Epic center (ER)	Suspend SC	>384	---	>340	August 2009
	Dragnet	>384	---	>207	

[a] Active ingredient in Suspend SC is Deltamethrin (0.06%); Active ingredient in Dragnet is Permethrin (0.05%)

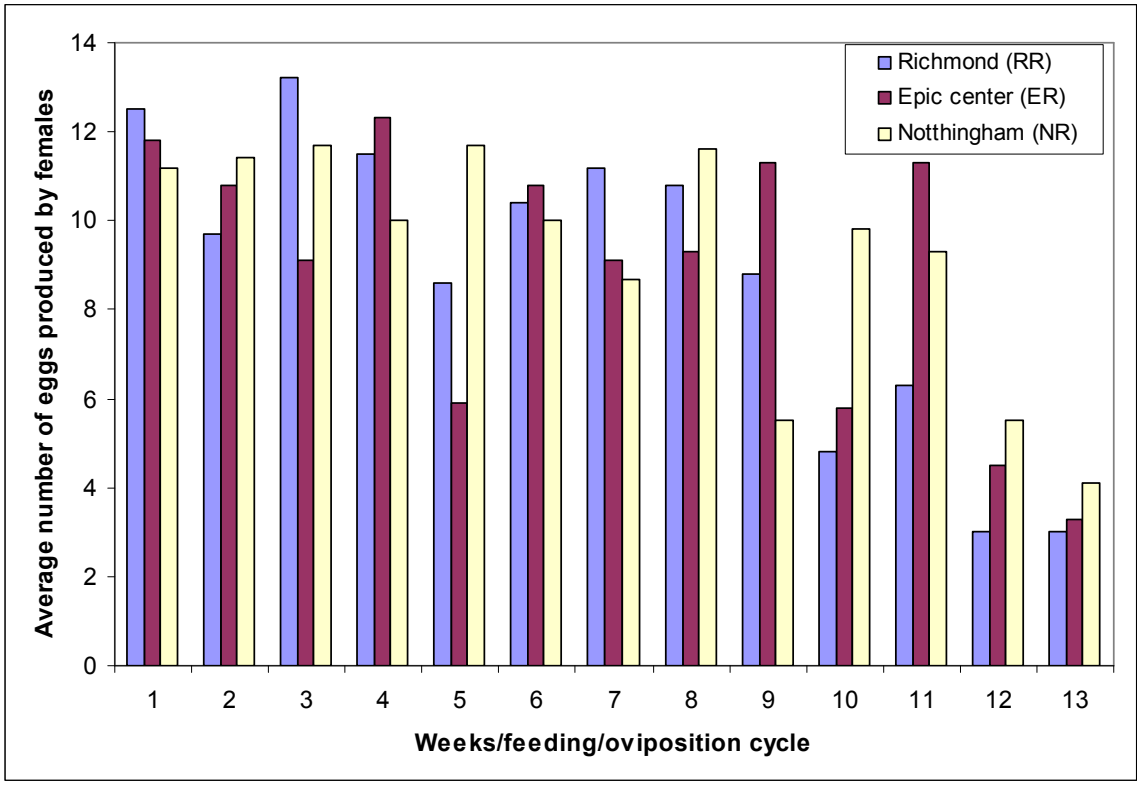

Figure 3.1. Average numbers of eggs laid per female from three field collected bed bug strains: Richmond, Epic Center and Nottingham Green throughout 13 feedings (~6 months).

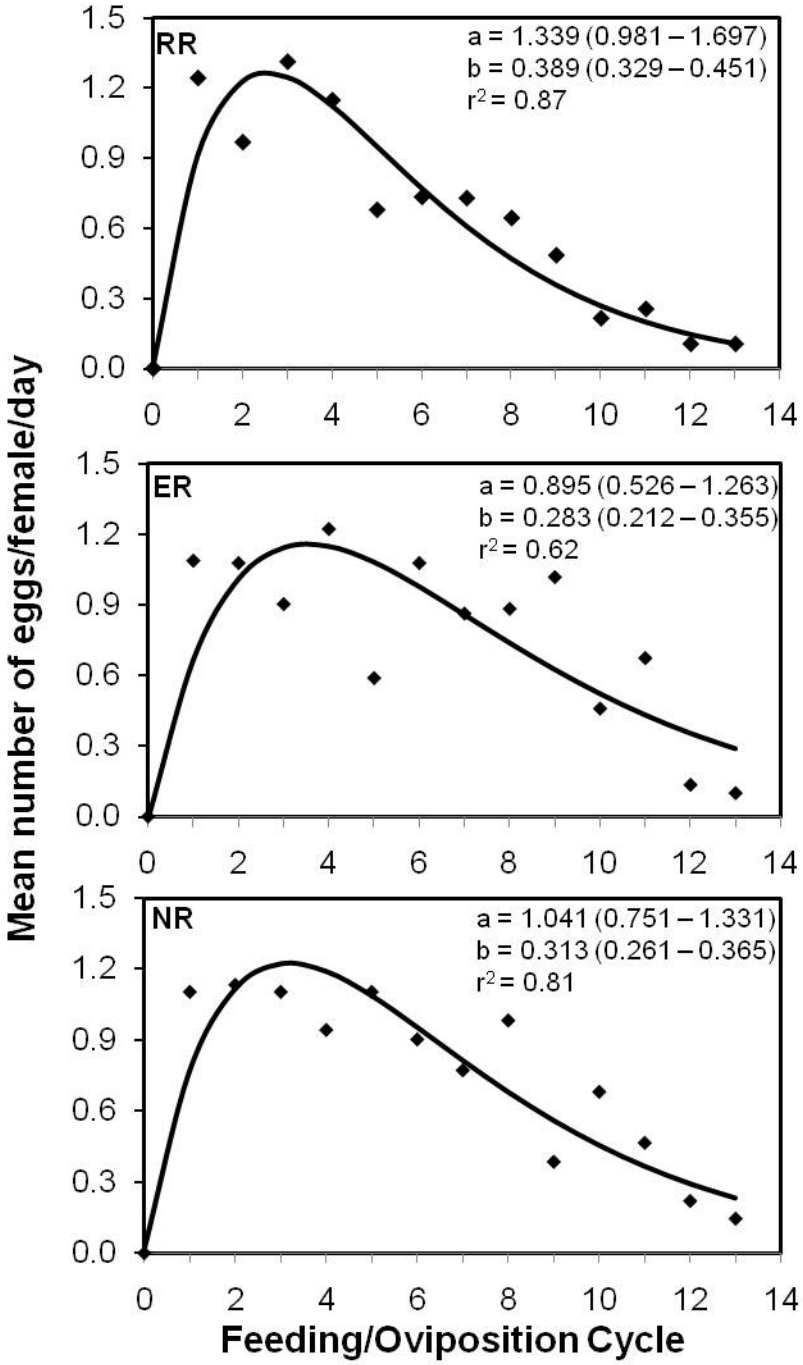

Figure 3.2. Fit of the model [$Y = aX \exp(-bX)$; solid line)] to data on the mean number of eggs/female/day (filled diamonds) for three strains of the common bed bug (Richmond - RR, Epic Center - ER, and Nottingham Green - NR) over the course of 13 feeding/oviposition cycles.

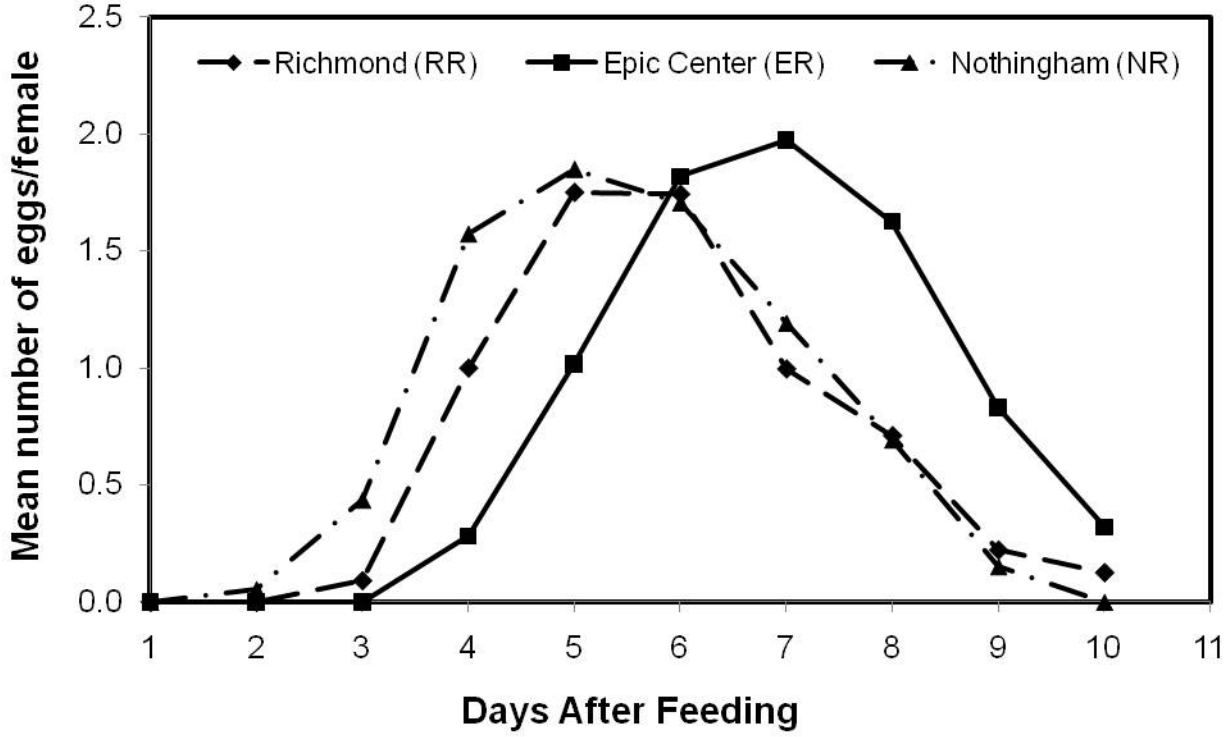

Figure 3.3. Patterns of oviposition between feedings for three stains of the common bed bug (Richmond - RR, Epic Center - ER, and Nottingham Green - NR) over the course of 13 feeding/oviposition cycles.

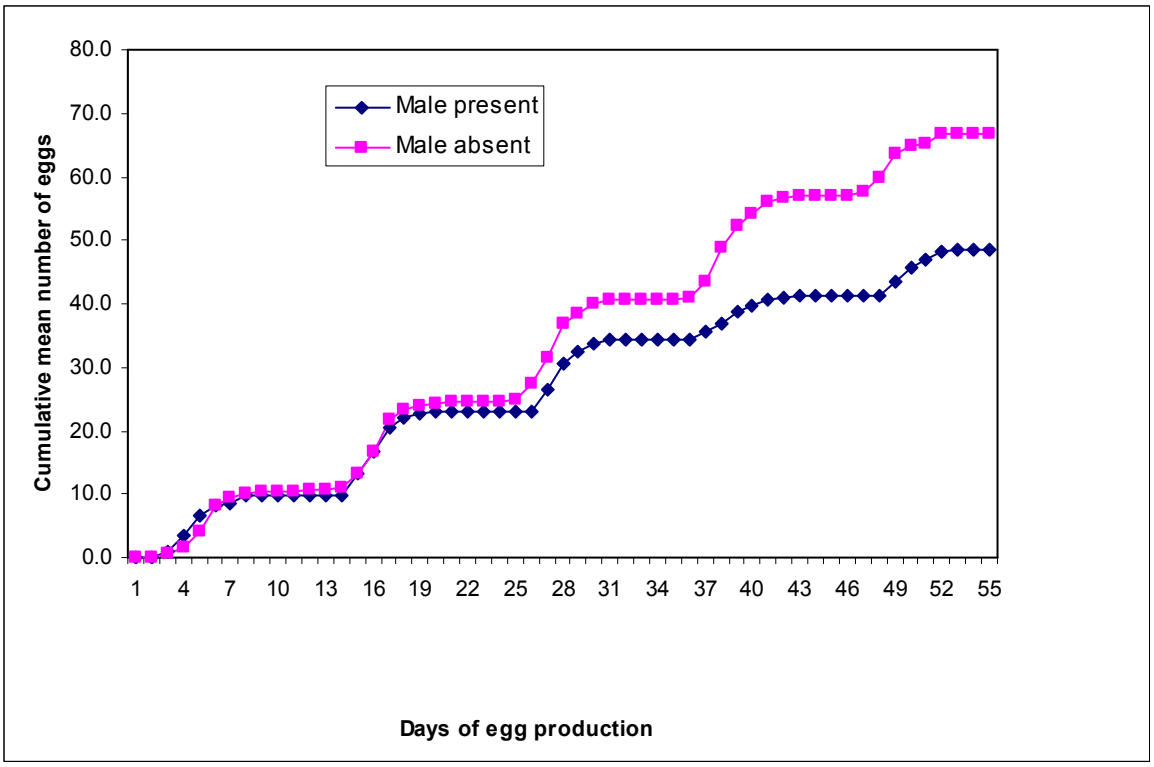

Figure 3.4. Cumulative mean number of eggs produced by 20 (RR) strain bed bug female mated only once or exposed to multiple matings over the course of five feeding/oviposition cycles.

Chapter 4: *C. lectularius* L. Survivorship during Starvation

Introduction

In recent years there has been a significant increase in bed bug infestations throughout the U.S. (Gangloff-Kaufmann et al. 2006, Cooper 2006). This increase in the U.S. is also an indicator of a more widespread increase across the globe (Potter 2010). The recent proliferation of bed bugs has been attributed to an increase in international travel (Boase 2001), people immigrating to and from other countries (Cooper 2006, Potter 2005), seasonal human migration (Kells 2006), lack of bed bug awareness (Cooper 2006), and bed bug resistance to insecticides such as pyrethroids (Potter 2006). Many pest management professionals in the U.S. have indicated that bed bugs are the most difficult pest they have had to deal with in their professional careers (Harlan 2006, Cabrera and Heinsohn 2006, Potter 2006).

The relationship between humans and bed bugs has been well documented throughout history (Usinger 1966), yet little is currently known of the recent biology and ecology of this species (Reinhardt and Siva-Jothy 2007). This lack of knowledge is due partly to the paucity of academic research over the last 50 years that resulted from the decline of bed bug populations in developed countries (Reinhardt and Siva-Jothy 2007). Most of the literature regarding the biology and ecology of the common bed bug, therefore, is somewhat outdated (e.g., Johnson 1941). For example, bed bug resistance to insecticides was either not recorded or unknown in biological studies conducted in the 1940s. Therefore, it is very likely that modern bed bug populations are at least somewhat different from the bed bug populations described in Johnson (1941) and Usinger (1966), particularly with respect to their fitness, specifically their survivorship.

The majority of insecticides currently used for bed bug control come from a single class, the pyrethroids. Repeated pyrethroid applications over time, however, have resulted in bed bug populations becoming resistant (Moore and Miller 2006, Potter 2006, Romero et al. 2007, Kyong et al. 2008). There are three types of physiological resistance that are well documented in insects. The first is reduced cuticular penetration by the insecticide. The second is the alteration of the target site or nerve cell membrane. The third is increasing or decreasing metabolic detoxification or sequestration (Mullins and Scott 1992, Soderlund and Bloomquist 1990). Many bed bug populations in the U.S. have been evaluated for resistance. All three types of resistance have been documented in *C. lectularius* partculary *kdr* type resistance (Yoon et al. 2008). Moore and Miller (2006) reported resistance to deltametrhin in a field strain of bed bugs collected in Virginia. Romero et al. (2007) also reported resistance to deltamethrin and lambda cyhalothrin in populations collected in California, Florida, Kentucky, Ohio, and Virginia.

The selection for genotypes that are insecticide resistant results in a reduction in fitness for the resistant population. Resistant populations invest more energy in insecticide detoxification than in reproduction and survivorship (Wood and Bishop 1981, Uyenomaya 1986, Roy et al. 2010). After exposure, surviving individuals with alleles that confer resistance may detoxify the insecticides. The detoxification of insecticides can interfere with the insect's original physiology specifically, their enzyme activity and function, reducing the insect's adult longevity (Uyenoyama 1986). Alternatively, the wasteful overproduction of an enzyme when there are no insecticide to detoxify may also play a role in reducing longevity as part of the insect's fitness (Clarke 1978). This reduction in fitness related to insecticide resistance has been documented in several insects including species in the families, Aphididae (Hollinsgsworth et al. 1997), Tortricidae (Carriere et al. 1994), Culicidae (Kumar et al. 2009), and Muscidae (Roush

and Plapp 1982, Scott et al. 1997, Krafsur et al. 1993). Even when no insecticide is present, resistant strains are found to be at a selective disadvantage because resistant alleles have negative effects in the individuals (Giorghiou and Taylor 1986).

The purpose of this study was to quantify the survivorship of all life stages of bed bugs (1th-5th and adults) during starvation. For this study, four bed bug strains with varying levels of resistance to pyrethroid insecticides were used (two pyrethroid-susceptible and two resistant strains) to determine differences in survivorship between strains during periods of extended starvation.

Materials and Methods

Bed bug rearing

A susceptible laboratory strain of bed bugs (HS) was acquired in February 2005 from Dr. Harold Harlan (National Pest Management Association, Fairfax, VA). Dr. Harlan maintained this population of bed bugs for >37 yr (since 1973). Two field strains were also collected. The Richmond strain (RR) was collected from apartments in Richmond, VA in September 2008. The Epic Center strain (ER) was collected in June 2008 from a hotel room in Cincinnati, OH. Finally, a British (BS) field strain was collected in September 2009 from an apartment in London, England. Both HS and BS are considered pyrethroid-susceptible strains, while RR and ER have been documented to possess high levels of pyrethroid resistance (Table 4.1).

The bed bug colonies were reared in plastic jars covered at one end with a cloth mesh. Two pieces of cardboard were placed inside of the jars so that bed bugs could crawl up the cardboard and stick their mouth parts through the cloth mesh to feed from an artificial feeder. An artificial feeder using circulating hot water maintained a diet of chicken blood at 35.5º C. Bed bugs were fed once a week on chicken blood formulated with sodium citrate as an anti-coagulant. Between feedings, bed bugs were stored in an environmental chamber at temperatures between 26.1º C and 26.5º C, 68.9% RH and photoperiod of 12:12 h, light and darkness. These conditions may be considered optimal for *C. lectularius* and closely approximated the conditions at which Johnson (1941) evaluated bed bug populations for maximum average fecundity and longevity. All bed bug colonies were maintained in the Dodson Urban Pest Management Laboratory at Virginia Tech, Blacksburg, VA.

Evaluation of survivorship during starvation

For this experiment the pyrethroid susceptible strains, HS and BS, and resistant strains, RR and ER, were selected for evaluation due to the fact that these strains came from very different source populations. Recently molted individuals from each developmental stage (1^{st}- 5^{th} instars and adults) were selected from each strain. The individuals were observed under the microscope to determine their developmental stage. Five replications with groups of 10 individuals from the same-stage individuals were placed in Petri dishes (Fisher brand 60 x 15 mm) containing a single filter paper (Whatman 42.5 mm). Adult males and females were also placed in separate Petri dishes to prevent copulation. A total of fifty individuals from each life stage were studied. No blood meals were offered to the different life stage groups' individuals after they were place inside the Petri dishes. The Petri dishes containing the bed bugs were checked daily and mortality was recorded. Mortality data was collected until all individuals had died.

Statistical analysis

Survivorship of each life stage and bed bug strain was compared by strain using ANOVA (JMP 8.0; SAS Institute 2005). Values of $P \leq 0.05$ were used to indicate significance. Means were separated using Tukey-Kramer HSD.

Results

Evaluation of survivorship after starvation

The survival time range over all strains for each life stage was 13.8 – 36.3, 27.8 – 68.3, 28.2 – 118.1, 33.4 – 124.2, 43.7 – 127.9, 43.7 – 92.6, 42.9 – 99.4 days for first, second, third, fourth, fifth instar nymphs, and for adult female, and male respectively (Table 4.2). In general, bed bug nymphs (1^{st}-5^{th} instars) from all the strains survived in average 60.37 ± 8.321 SE days, while adults (female and male) survived 69.81 ± 7.47 SE days.

Survival time after starvation for the HS strain was significantly shorter for the 1st and 2^{nd} instars compared to the other developmental stages. Third, fourth, and fifth instar nymphs, and adult male and female's survival time during starvation was not significantly different (Table 4.2).

Survival time after starvation for the BS strain was significantly shorter for the 1st and 2^{nd} instars compared to the other developmental stages. There were no significant differences in survival time between third, fouth, and fifth instars; however, their survival time was significantly longer when compared to adult male and female life stages (Table 4.2).

Survival time after starvation for the ER strain was significantly shorter for the 1^{st}, 2^{nd} and 3^{rd} instars compared to the other developmental stages. There were no significant differences in survival time between 4^{th}, 5^{th} and adult male and female life stages (Table 4.2).

Survival time after starvation for the RR strain was significantly shorter for the 1^{st} instar compared to the other developmental stages. There were not significant differences in survival time between 2^{nd} and 3^{rd} instars; however, their survival time was significantly shorter when compared to 5^{th} instar and adult male and female life stages (Table 4.2).

Overall, mean survival time during starvation differed significantly ($F = 258.23$; df = 3, 1396; $P < 0.0001$) between the four bed bug strains. The Richmond strain was the most susceptible to starvation of the resistant strains. Resistant strains presented significantly shorter ($P < 0.0001$) survival time than susceptible strains for each developmental stage. Mean survival time during starvation for the nymphal stages was 93.4 ± 19.4 SE days for the BS strain followed by HS with 75.6 ± 12.4 SE days. The RR resistant strain presented the shortest survival time 29.4 ± 4.8 SE days followed by ER 43.1 ± 10.7 SE days (Figure 4.1). Mean survival time during starvation for the adult stages was 96.0 ± 3.4 SE days for the HS strain followed by BS with 78.6 ± 4.1 SE days. The RR resistant strain presented the shortest survival time 43.3 ± 0.4 SE days, followed by ER with 61.4 ± 1.1.

Discussion

Our study suggests that susceptible bed bug populations can survive longer periods of starvation than pyrethroid resistant populations. First instar bed bugs from all the strains had a shorter survival time when compared with the other life stages. Our study may help to clarify or update some information about bed bug survivorship during prolonged periods of starvation. It is very common to find information online describing bed bug populations living for several months (even years) without a blood meal. For instance, BadBedBug.com states in their website: "bed bug adults can survive more than 12 months without feeding". Bed Bugs.org states: "adult bed bugs can survive for up to 7 months without a blood meal and have been known to live in empty buildings for up to one year". Long periods of bed bug survival time during starvation have been also reported in universities' extension fact sheets. The University of Minnesota's extension web site quotes: "immature bed bugs may live for several months without feeding while adults may survive as long as one year". The Ohio State University extension fact sheet quotes: "The bed bugs adult's lifespan may encompass 12-18 months".

Survivorship in *C. lectularius* has been studied by many researchers (Bacot 1914, Kemper 1930, Johnson 1941, Omori 1941, Gunn 1933) under different environmental conditions. However, each study provided different results regarding how long a bed bug can live without a blood meal. Kemper (1930) found that from all of the stages the 5th instar and adult males live longer during starvation than the other life stages. The study developed at 22°C and 40-45% RH showed fifth instar and adult males living for 141.8 and 142.6 days during starvation. Johnson (1940) reported a female bed bug living for more than 580 days after the last blood meal. Bacot (1914) reported keeping starved bed bugs from all developmental stages at 24°C for 18 months. After 18 months of starvation the bed bugs were even able to feed again.

Gunn (1933) reported adult bed bugs living for more than three years with sporadic feedings (approximately 23 feedings over that period of time). However, the temperature and relative humidity at which the experiment was developed were not stated. The results of this study were quite different from those reports prior to 1950. In fact, the highest mean survivorship after starvation for 5th instar, adult male and adult female bed bugs was 128 d, 99.4 d, and 92.6 d, respectively. The maximum survivorship was recorded for a 5th instar bed bug from the BS strain, which lived 135 days without a blood meal.

In this study, we found that first instar bed bugs die faster than the other developmental stages (13.8-36.3 d mean survival time). Other studies also reported the first instar as the most susceptible to starvation (Kemper 1930, Johnson 1941 and Usinger 1966). One explanation for high mortality in first instar bed bugs is undoubtedly moisture loss. Recently hatched bed bugs lose moisture easily, because they have a larger surface area compared to their volume ratio (Usinger 1966). In our study, first instar bed bugs from all the four strains survived an average 23.4 ± 5.5 d during starvation, while Kemper (1930) reported a survivorship of 83.7 days after starvation at 22°C and 40-45% RH for first instar bed bugs.

We also found that adult male and female bed bugs in each strain survived the same amount of time after starvation (43.7-92.6 d for adult females and 42.9-99.4 d for adult males). Omori (1941) stated that virgin adult females lived longer than males during starvation (43.4 d for males and 86.7 d for females). Johnson (1941) and Kemper (1930) also reported virgin females lived longer than males. Omori (1941) found that at 27°C, 70-75% RH third instar bed bugs survived on average 71.2 days after the last blood meal. However, we found that third instar bed bugs from the field strains ER and RR survived no longer than 38 days after the last blood meal.

In this study, bed bugs from the resistant strains died more repidly than bed bugs from susceptible strains during starvation. The fact that we found significant differences in the survivorship between resistant and susceptible strains may suggest that resistance to insecticides may play a role in the bed bug's survivorship during starvation. We cannot make specific predictions about the survivorship of different populations of *C. lectularius*; however, it is very interesting to note that the two susceptible strains lived significantly longer than the resistant bed bug strains. The significant differences in survivorship during starvation between susceptible and resistant strains may be the consequence of a fitness trade-off in resistant populations.

Populations of insects resistant to insecticides are known to have lower fitness than susceptible populations (Georghiou and Taylor 1986). Groeters et al. (1994) reported that resistant populations of diamondback moth (*Plutella xylostella*) had shorter lives than susceptible populations. Carriere et al. (1994) also found that resistant populations of *Choristoneura rosaceana* (Tortricidae) had higher mortality than the susceptible populations. Kumar et al. (2009) found a significant reduction in longevity in a strain of *Aedes aegypti* resistant to deltamethrin (a common pyrethroid used for bed bug control) than in susceptible populations. Rosenheim and Tabashnik (1990) simulated a model where fitness was measured for a hypothetical insecticide resistant insect. Rosenheim and Tabashnik (1990) predicted a significant fitness cost in resistant strains even in the absence of the insecticide.

This study was the first to document differences in survivorship during periods of starvation in bed bug populations that are known to be pyrethroid resistant. However, more studies are needed to quantify differences between populations in response to starvation.

Table 4.1. Time to mortality of adult bed bugs (50% male: 50% female) confined on hardboard panels treated with residual insecticide. Samples run same day for comparison.

Bed Bug Strain	Treatment[a]	LT_{50} (h)	95% CLs	Resistant ratios	Test Date
Harlan (HS)	Suspend SC	0.8	0.72–0.94	---	February 2009
	Dragnet	1.5	1.23–1.78	---	
Richmond (RR)	Suspend SC	320.2	304.4–338.2	390.5	February 2009
	Dragnet	>432	---	291.7	
Harlan (HS)	Suspend SC	0.5	0.47–0.54	---	July 2010
	Dragnet	3.9	3.67–4.19	---	
British (BS)	Suspend SC	1.3	1.30–1.37	2.6	July 2010
	Dragnet	3.3	2.65–3.89	0.8	
Harlan (HS)	Suspend SC	1.1	1.06–1.19	---	August 2009
	Dragnet	1.9	1.57–2.13	---	
Epic center (ER)	Suspend SC	>384	---	>340	August 2009
	Dragnet	>384	---	>207	

[a] Active ingredient in Suspend SC is Deltamethrin (0.06%); Active ingredient in Dragnet is Permethrin (0.05%).

Table 4.2. Comparison of mean ± (95% CI) survival time (days) during starvation of immature and adult life stages of two pyrethroid-susceptible (HS and BS) and two pyrethroid-resistant (RR and ER) bed bug strains (n = 50 individuals in each life stage).

Bed Bug Strain	**Mean Survival Time (d) after Starvation (95% CI)**						
	Instar					**Adult**	
	1st	**2nd**	**3rd**	**4th**	**5th**	**Female**	**Male**
Harlan (HS)	36.3 **a** (33.7–38.9)	56.3 **b** (51.9–60.7)	90.1 **c** (84.7–95.5)	95.8 **c** (89.8–101.8)	99.3 **c** (92.4–106.3)	92.6 **c** (85.6–99.7)	99.4 **c** (93.3–105.5)
British (BS)	28.6 **a** (25.9–31.2)	68.3 **b** (63.9–72.7)	118.1 **c** (112.7–123.6)	124.2 **c** (118.2–130.3)	127.9 **c** (120.9–134.9)	74.5 **bd** (67.5–81.6)	82.6 **d** (76.5–88.6)
Epic Center (ER)	15.0 **a** (12.4–17.6)	27.9 **b** (23.6–32.4)	38.3 **c** (32.9–43.7)	61.0 **d** (54.9–67.0)	73.3 **d** (66.2–80.1)	62.5 **d** (55.5–69.6)	60.3 **d** (54.2–66.4)
Richmond (RR)	13.8 **a** (11.2–16.4)	27.8 **b** (23.5–32.2)	28.2 **b** (22.8–33.6)	33.4 **bc** (27.3–39.4)	43.7 **c** (36.8–50.7)	43.7 **c** (36.6–50.7)	42.9 **c** (36.9–49.0)

* Different letters in the same row indicate significant differences ($P \leq 0.05$).

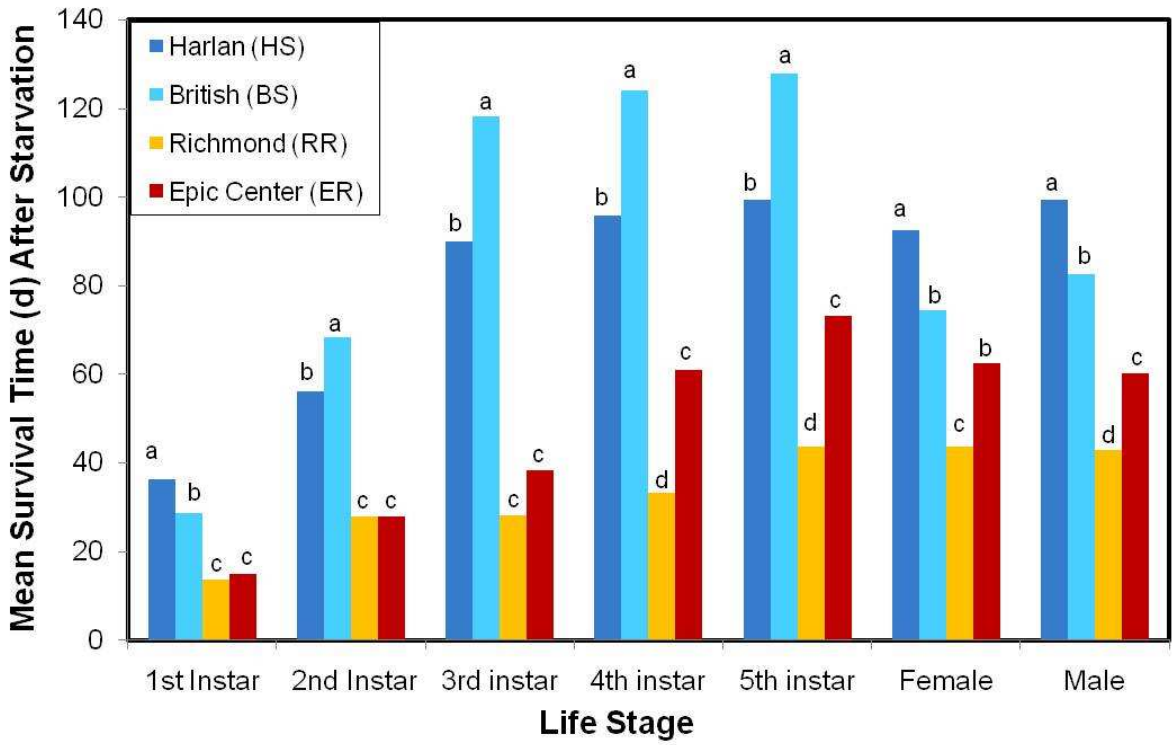

Figure 4.1. Comparison of life stage survival during starvation between pyrethroid-susceptible (HS and BS) and pyrethroid-resistant (RR and ER) strains of the common bed bug (n = 50). Different letters above bars indicated significant differences in mean survival time within the life stage ($P < 0.05$).

Chapter 5: Developmental and Reproductive Life Tables for the Common Bed Bug, *C. lectularius* L.

Introduction

During the 1940's, a new synthetic organochloride insecticide, dichlorodiphenyl trichloroethane (DDT), was developed primarily to control insect vectors of diseases such as malaria and typhus among military and civilian populations (Potter 2008). Because of its long residual activity and low cost, DDT was also used for controlling populations of other human insect pests, including the common bed bug, *Cimex lectularius* (Usinger 1966). The widespread use of DDT in the 20th century essentially eradicated bed bug populations from the U.S (Potter 2008, Harlan 2006, Harlan 2007, Cooper 2006). However, beginning in the 1990's, there have been growing reports of bed bug infestations in the United States. Pest management companies have experienced exponential increases in complaints between the years 2000 and 2008 (Potter 2008).

The majority of insecticides that were used for bed bug control in the 1950s and 1960s are no longer available for use today. DDT, organophosphate, and carbamate insecticides used for bed bug control were discontinued by the Environmental Protection Agency (EPA) primarily because of their potential toxicity to the environment and human health (Potter 2006). The majority of insecticide products that are currently available for bed bug control belong to the pyrethroid class.

Repeated applications of pyrethroids over time have led to the development of pyrethroid resistance in bed bugs. Moore and Miller (2006) studied a field strain of bed bugs resistant to pyrethroids and found that these insects can survive long periods of exposure (as much as 343 hours) to dried residues on treated panels. Prior to their resurgence in the U.S., bed bug

infestations in other countries were treated with both DDT and pyrethroids. Therefore, the resistance of the bed bug to pyrethroids may be the result of the selection pressure experienced in other countries (Romero et al. 2007). In addition, the continued use of pyrethroids in the U.S. to control resistant populations has further increased the potential prevalence of the alleles responsible for the resistance in bed bug populations (Romero et al. 2007, Yoon et al 2008) making it even more difficult to control this pest.

In order to control modern bed bug populations, a better understanding of their ecology and biology of the insect is required. The biological characteristics of *C. lectularius* was described between the 1930s and 1960s (e.g., Johnson 1940, Usinger 1966), as were the most significant aspects of the ecology of the insect (e.g., Gunn 1933, Johnson 1941, Davis 1964). While all of the biological and ecological information collected during the first decades of the 20th century still has value, it may be unwise to assume that the bed bug populations currently infesting the United States are exactly the same as those that existed a century ago.

Effective insect pest management strategies require that the pest manager have a good understanding of the pest population. Models that describe pest populations are useful in designing pest management strategies (Pedigo 1996). One mechanism for understanding and describing population characteristics are life tables. Life tables measure the biological attributes of the insect population such as mortality, survivorship and fecundity in a population (Krebs 2001). In addition, life tables can be used to determine the reproductive contribution that adults make to future generations. Life tables can also be used to calculate the maximal rate of increase at any particular combination of environmental factors, such as temperature and humidity (Krebs 2001).

The purpose of this study was to document and quantify the biological attributes of selected bed bugs strains. These attributes included the development and survivorship of the immature stages, and adult survivorship and fertility. These biological attributes were summarized in the form of a cohort life table to reflect the stage transition dynamics (development, survivorship, and reproduction) of the insect under specific environmental conditions.

Materials and Methods

Bed bug rearing

A laboratory bed bug strain (HS) was acquired from Dr. Harold Harlan (National Pest Management Association, Fairfax, VA) in February 2005. Dr. Harlan had maintained this population of bed bugs for >37 yr (since 1973 feeding them on himself). Two other bed bug strains were collected from the field. The first field strain, Kramer (KR), was collected in 2006 from a multiple unit apartment building in Arlington, VA. The other field strain, Richmond (RR), was collected in September 2008 from a group home for adult residents in Richmond, VA. HS is considered pyrethroid-susceptible strains, while KR and RR have been documented to possess high levels of pyrethroid resistance (Table 5.1). All bed bug colonies were maintained in the Dodson Urban Pest Management Laboratory at Virginia Tech, Blacksburg, VA.

The bed bug colonies were reared in plastic jars covered at one end with a cloth mesh. Two pieces of cardboard were placed inside of the jars so that bed bugs could crawl up the cardboard and stick their mouth parts through the cloth mesh to feed. Bed bugs were fed once a week on chicken blood formulated with sodium citrate as an anti-coagulant. An artificial feeder using circulating hot water maintained the chicken blood at 35.5º C. Between feedings, bed bugs were stored in an environmental chamber at temperatures between 26.1º C and 26.5º C, 68.9% RH and photoperiod of 12:12 h, L:D. These conditions may be considered optimal for *C. lectularius* and closely approximated the conditions at which Johnson (1941) evaluated bed bug populations for maximum fecundity and longevity.

Evaluation of bed bug survivorship (life table)

Male and female fifth instars were selected from each bed bug strain (HS, KR and RR). Individuals were fed and allowed to molt to the adult stage. The process of feeding was carried out by inverting the fifth instar nymphs in their respective jars and placing the mesh top against the arm of a human volunteer. The process of feeding was approved by the Virginia Tech Institutional Review Board (IRB 06-165). Once the fed 5^{th} instars molted into adults, they were sorted into couples (one male and one female) and fed again on human blood as described above. Once fully engorged, the couples were placed in Petri dishes (Fisher brand 60 x 15 mm) containing a single filter paper (Whatman 42.5 mm) and left to produce eggs. Eggs produced by all couples on the same day were grouped into cohorts and their development was observed and recorded through adulthood. Egg hatch, mortality, and molting were observed and recorded daily. The developmental times (number of days from one stage to the next and from hatch to adulthood) were recorded for each individual.

Evaluation of bed bug fecundity (life table)

Adult males and females from the RR field strain were placed in individual pairs in Petri dishes (Fisher brand 60 x 15 mm) containing filter papers (Whatman 42.5 mm). The couples were fed on human blood as described above and then left to produce eggs. The number of eggs produced were recorded daily until egg laying ceased and the adults were given another blood meal. Adults were fed approximately every 7 days and egg laying was quantified until the adult female died. Reproduction (mean number of offspring produced through the entire lifetime), adult lifespan (number of days from first reproduction to death), and total lifespan (number of days from hatch to death) were recorded for each adult bed bug.

Survivorship and fecundity life tables

The survivorship data collected for resistant field strains (RR and KR), and the laboratory susceptible strain (HS), were used to generate survivorship life tables (egg to adulthood) using the procedures described in Carey (1993). The survivorship life table parameters used in this study were: n_x (number of individuals at age interval *x)*, l_x (fraction surviving at the beginning of age interval *x*), p_x (finite rate of survival), q_x (finite rate of mortality), d_x (fraction dying at age interval *x)*, L_x (mean number of individuals alive), T_x (number of days lived beyond age *x)*, and e_x (mean expectation of life).

In addition, the data collected from the evaluation of bed bug fecundity was used to develop a complete (survivorship and fertility) life table for the resistant field strain RR using the methods described by Leslie and Park (1949). Several additional life history parameters were derived from this life table. These included R_o, the average number of offspring produced by an individual female in her lifetime calculated as $R_o = \sum(l_x m_x)$, where l_x was the age-specific survivorship and m_x was the age-specific fecundity. An estimate of the per-capita intrinsic growth rate *r*, defined as the number of progeny born to each female bed bug per unit of time, was calculated as $r = \ln(R_o)/G$, where $G = \sum l_x m_x x / \sum l_x m_x$, and *x* was bed bug age. A more precise estimate of r was calculated iteratively using the relationship $\sum e^{-rx} l_x m_x = 1.0$.

Certain assumptions were made in developing the survivorship and fecundity life tables. It was assumed that all of the eggs laid were fertile and that failure to hatch was due to embryonic mortality. It was impossible to determine time of death in the egg stage, and the total mortality for this part of the life cycle was therefore assumed to be distributed evenly over the entire period. Because we followed the transition of eggs to adult stage, the survivorship life

tables ended with the development of the 5th instars into the adult stage or their mortality. Finally, the sex of individual bed bugs could not be determined in the egg or immature stages, and it was therefore assumed that the mortality rates in these stages were equally applicable to males and to females.

Data and statistical analysis

The data in the life tables were used to derive estimates of the bed bug stage-specific developmental duration. Mean (± 95% CI) duration of development for each of the five immature stages of the insect, egg, 1st instar (N1), 2nd instar (N2), 3rd instar (N3),4th instar (N4), and 5th instar (N5), were estimated using the methods described in Pontius et al. (1989) and Carey (1993). Significant differences within each life stage among the three bed bug strains were determined by the non-overlapping confidence intervals.

Results

Bed bug survivorship

The egg to adult survivorship life table for HS, KR, and RR bed bug strains are presented in Tables 5.2, 5.3, and 5.4, respectively. Several biological differences between the three strains are evident in the life table. Survivorship (l_x) to the adult stage was relatively high for the HS (≈80%) and RR (94%) strains. However, survivorship was much lower in the KS strain (69%). Survivorship in the egg stage was extremely high (from 100% to 96%) for all three strains. The KS strain suffered greatest nymphal mortality during N2 (2nd instar) stage relative to the other two strains. Mortality from egg to the adult stage varied from 6% for the RR strain to ~21% and 31% for the HS and KS strains, respectively.

Another interesting difference among the three strains was found by examining the e_x column of each life table. The expectation of life (the number of per capita days of life remaining to the average individual) for an egg laid by a female of the HS, KS, and RR strain before it enters the adult stage was 35.3, 32.2, and 34.4 d, respectively. This means that any egg from the HS and RR strains is expected to be alive approximately 2–3 days longer before entering the adult stage compared with an egg of the KS strain. This pattern of life expectancy changes when the egg enters the N1 stage. Tables 5.2, 5.3, and 5.4 show that once an egg reaches the N1 stage the amount of life it has remaining before becoming an adult differs among the strains with the HS, KS, and RR strains having e_x values of 32.6, 29.9, and 27.7 d, respectively. The data suggest that for the HS and KS strains, life expectancy increases as an individual makes the transition from the egg to N1 stage. On the other hand, life expectancy for an individual of the RR strain decreases for the same transition, and remains relative low at the transition point between other immature stages compared with HS and KS.

Developmental time and survivorship among bed bug strains

Data on the duration of development for the immature life stages are presented in Table 5.5. There was no significant difference ($P > 0.05$) between the development times for the HS and KS strains. However, the development times for these two strains differed significantly ($P < 0.05$) from those of the RR strain. The RR strain had a significantly longer developmental duration in the egg and N5 stages, and significantly shorter developmental duration in the N1, N2, N3, and N4 stages compared with the HS and KS strains. Overall, the mean duration of development from egg to adult was significantly shorter for the RR strain compared with the other two strains.

Fecundity of RR bed bug strain

The fertility life table for Richmond strain bed bugs is presented in Table 5.6. The average daily output of eggs by a single female during her entire lifetime was 0.65 which roughly equates to a female producing one egg every two days. However, the highest m_x value (2.075) was observed at 78 d of adult age (Table 5.6).

The mean (±SE) duration of adult life (from the day a female becomes an adult until the day she dies) was 156.4 ± 12.4 with standard deviation of 55.5 d. Six females survived as adults to 227 days. The range of life length for all tested females (egg to adult) was 81–227 days. However, the expectation of life of a newly laid egg until death as an adult was approximately 142.5 d, with a newly molted adult having an expectation of life of about 125 d (Table 5.6).

The maternal frequency (m_x) shows the average number of female eggs produced per day by a female alive at age x. The sum of this column, 53.3, represents the gross reproductive rate,

or the average number of daughter eggs that would be expected to be produced by a female living throughout her entire reproductive period. The average daily output of daughter eggs by such a female is 0.65. The sum of $l_x m_x$, 35.1 is the net reproductive rate, R_o (Table 5.7). R_o indicates that one live female egg would on the average be replaced by approximately 35 live daughter eggs, that is, there is a 35-fold increase of females per generation. The approximate value of the intrinsic rate of increase of *C. lectularius* was $r = 0.0386$. The more precise r-value was found to 0.539. The mean length of a generation, given by $G = \log_e R_o/r$, was 92.31 d (Table 5.7). Therefore, a population increasing at the rate of 0.05 per individual per day would double in number every 12.86 d.

Discussion

The first purpose of this study was to assess the differences in survivorship, developmental time and mortality between three bed bug strains with different levels of resistance to pyrethroids. In our study the RR resistant strain survived longer from egg to adulthood on a regular feeding schedule than the susceptible HS. This is interesting because most resistant populations of insects have a reduction in longevity due to the amount of energy invested in insecticide detoxification (Georghiou and Taylor 1986, Uyenoyama 1986). In addition, the life expectancy or the amount of life that RR individuals have remaining before becoming adults, was higher than the life expectancy of the other two bed bug strains. In our study, the RR strain developed faster from egg to adulthood than the HS strain. Banks and Needham (1970), also reported a shorter mean duration of nymphal stages in resistant aphid populations relative to susceptible populations. However, several other studies have found that resistant populations have longer developmental time than susceptible populations (Roy et al. 2010, Georghiou and Taylor 1977 and Carriere et al. 1994). Roy et al. (2010) observed that nymphs in a resistant population of tea mosquito bug (Miridae) took longer to develop than susceptible nymphs. Ferrari and Georghiou (1981) also reported longer developmental times for resistant strains of southern house mosquitos (Culicidae), relative to susceptible populations.

Most studies indicate that resistant populations of arthropods have a longer developmental time than susceptible populations (Georghiou and Taylor 1977 and Carriere et al. 1994). There is often a trade-off between the alleles that confer resistance and the alleles that determine the fitness in resistant populations of insects (Groeters et al. 1994). This trade off is because much of the insect's energy is invested in producing detoxification enzymes rather than in development (Roush and Daly 1990). We cannot explain why resistant bed bugs in our study

developed faster than the susceptible bed bugs. However, we can suggest that the superior nymphal survivorship and rapid development does confer the advantage of reducing time to reproduction. However, more studies need to be conducted to confirm that these differences between resistant and susceptible bed bugs are consistent among different populations.

Another goal of this study was to develop a reproductive life table for a field strain (RR) of *C. lectularius* to understand the life history of modern populations. The present investigation provides new detailed information on the egg production and length of modern bed bug populations currently infesting the United States. RR females lived an average of 159.7 days, however, the standard deviation was 52.8 days. The standard deviation clearly indicates that the lifespan in the population is very variable. For example, some females were producing their maximum number of eggs (m_x = 2.08) at 78 d of age, while others were dying at 78 days of age. We also found that during her lifetime, a bed bug female can produce an average of 85.75 eggs, or one egg every two days. The maternal frequency (m_x) calculation suggests that a female can produce an average of 54 daughters throughout her reproductive period. From those 54 daughters, about 35 will survive and reproduce to start a new generation of bed bugs.

Our study determined that the average adult life length was 160 days. Gunn (1933) reported adult bed bugs surviving for 3–4 years with only occasional feedings. Geisthardt (1937) reported that regularly fed adult bed bugs survived for more than 326 days at 22 ºC. The current study found that adult female bed bugs from the RR strain survived on average 159.7 days at 26 ºC and 69% RH with regular feedings (every~10 days). It would appear that the bed bug population in the Johnson (1940), Gunn (1933) and Geisthardt (1937) studies lived longer than the RR strain bed bugs. One of the reasons for this difference might be that the strains from 1930's and 1940's were not insecticide resistant. Groeters et al. (1994) reported that resistant

Hawaiian strains of *Platella xylostella* (diamondback moth) had a shorter life span than susceptible strains.

The intrinsic rate of increase (*r*) in a population was defined by Birch (1948) as the rate of increase per individual under specific conditions. In the laboratory it is easy to obtain optimal conditions for a specific species' development. Therefore, the intrinsic rate of increase obtained from a laboratory experiment can represent the maximal or nearly maximal rate of increase (Evans and Smith 1952). Small *r* values represent species growing slowly, and high *r* values represent species growing at a fast rate. For example, *Escherichia coli*, a species of bacteria that can increase on average 120-fold every 21 minutes (Delbrück, 1946), has an *r* value of 328, while some species of cicada that live for more than 17 years have an *r* value less than 0.001. Bed bugs from our experiments had an intrinsic rate of increase of 0.054, which means that the bed bug population is increasing at a rate of 0.054 individuals per female per day.

The *r* value calculated for bed bugs is quite impressive when compared with that of the German cockroach *Blattella germanica*, which has an *r* value of 0.06 (Aguilera et al 1997). German cockroaches are considered prolific reproducers and are very successful at infesting human environments. Current bed bug populations appear to be equally successful at proliferating in homes and apartments. Our study also found that populations of bed bugs can double in number approximately every 13 days. It is for this reason that an early detection is so important to prevent an infestation. If an infestation goes unnoticed, every 13 days the population will double in number.

The practical value of the life tables is to provide information about the life history of the species and aid in the development of control measures (Morris and Miller 1954). With the present study, we found that for the common bed bug life history parameters are related to the

level of resistance in the populations. This is the first time a complete life table has been developed for *C. lectularius*. Although this study represents a first step into characterizing modern bed bug populations that are infesting the United States, it will be necessary to develop more life tables in the future to characterize additional field strains. By improving our knowledge of the biological characteristics of these populations we may be able to design prevention measures to inhibit the bed bugs' proliferation, and reduce their current rate of spread.

Table 5.1. Time to mortality of adult bed bugs (50% male: 50% female) confined on hardboard panels treated with residual insecticide. Samples run same day for comparison.

Bed Bug Strain	Treatment[a]	LT_{50} (% h)	95% CLs	Resistance ratios	Source
Harlan (HS)	Suspend SC	1.0	1.00–1.15	---	Moore and Miller (2006)
	Dragnet	1.5	1.37–1.56	---	
Kramer (KR)	Suspend SC	>343	328.30–358.80	339.6	Moore and Miller (2006)
	Dragnet	>168	---	115.1	
Harlan (HS)	Suspend SC	0.8	0.72–0.94	---	Unpublished data (February 2009)
	Dragnet	1.5	1.23–1.78	---	
Richmond (RR)	Suspend SC	320.2	304.40–338.18	390.5	Unpublished data (February 2009)
	Dragnet	>432	---	291.7	

[a] Active ingredient in Suspend SC is Deltamethrin (0.06%); Active ingredient in Dragnet is Permethrin (0.05%).

Table 5.2. Survivorship life table for the immature bed bug stages of a pyrethroid-susceptible laboratory strain (Harlan, HS).

Age Class (Days), *x*	Age Interval (days)	Life Stage[a]	Number Alive at Start of Age Interval x, n_x	Fraction Living at Age *x*, l_x	Fraction Surviving from *x* to *x* +1, p_x	Fraction Dying from x to x +1, q_x	Fraction Dying in Interval *x* to *x* +1, d_x	Days lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x
0	0–1	Egg	175	1.00	1.00	0.00	0.00	1.00	35.31	35.31
1	1–2		175	1.00	1.00	0.00	0.00	1.00	34.31	34.31
2	2–3		175	1.00	1.00	0.00	0.00	1.00	33.31	33.31
3	3–4		175	1.00	0.99	0.01	0.01	0.99	32.31	32.31
4	4–5		173	0.99	0.97	0.03	0.03	0.97	31.32	31.68
5	5–6		168	0.96	0.94	0.06	0.06	0.93	30.34	31.61
6	6–7	N1	158	0.90	0.97	0.03	0.02	0.89	29.41	32.58
7	7–8		154	0.88	0.99	0.01	0.01	0.88	28.52	32.41
8	8–9		153	0.87	1.00	0.00	0.00	0.87	27.64	31.62
9	9–10		153	0.87	1.00	0.00	0.00	0.87	26.77	30.62
10	10–11		153	0.87	1.00	0.00	0.00	0.87	25.89	29.62
11	11–12		153	0.87	0.98	0.02	0.02	0.87	25.02	28.62
12	12–13		150	0.86	0.99	0.01	0.01	0.85	24.15	28.18
13	13–14		148	0.85	1.00	0.00	0.00	0.85	23.30	27.55
14	14–15	N2	148	0.85	1.00	0.00	0.00	0.85	22.46	26.55
15	15–16		148	0.85	1.00	0.00	0.00	0.85	21.61	25.55
16	16–17		148	0.85	0.99	0.01	0.01	0.84	20.77	24.55
17	17–18		146	0.83	1.00	0.00	0.00	0.83	19.93	23.88
18	18–19		146	0.83	1.00	0.00	0.00	0.83	19.09	22.88
19	19–20		146	0.83	1.00	0.00	0.00	0.83	18.26	21.88

Age Class (Days), x	Age Interval (days)	Life Stage[a]	Number Alive at Start of Age Interval x, n_x	Fraction Living at Age x, l_x	Fraction Surviving from x to x +1, p_x	Fraction Dying from x to x +1, q_x	Fraction Dying in Interval x to x +1, d_x	Days lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x
20	20–21		146	0.83	1.00	0.00	0.00	0.83	17.42	20.88
21	21–22		146	0.83	0.99	0.01	0.01	0.83	16.59	19.88
22	22–23	N3	144	0.82	1.00	0.00	0.00	0.82	15.76	19.15
23	23–24		144	0.82	0.99	0.01	0.01	0.82	14.94	18.15
24	24–25		143	0.82	1.00	0.00	0.00	0.82	14.12	17.28
25	25–26		143	0.82	1.00	0.00	0.00	0.82	13.30	16.28
26	26–27		143	0.82	1.00	0.00	0.00	0.82	12.48	15.28
27	27–28		143	0.82	1.00	0.00	0.00	0.82	11.67	14.28
28	28–29		143	0.82	0.99	0.01	0.01	0.81	10.85	13.28
29	29–30		141	0.81	1.00	0.00	0.00	0.81	10.04	12.46
30	30–31	N4	141	0.81	1.00	0.00	0.00	0.81	9.23	11.46
31	31–32		141	0.81	1.00	0.00	0.00	0.81	8.43	10.46
32	32–33		141	0.81	1.00	0.00	0.00	0.81	7.62	9.46
33	33–34		141	0.81	1.00	0.00	0.00	0.81	6.81	8.46
34	34–35		141	0.81	1.00	0.00	0.00	0.81	6.01	7.46
35	35–36		141	0.81	1.00	0.00	0.00	0.81	5.20	6.46
36	36–37		141	0.81	1.00	0.00	0.00	0.81	4.40	5.46
37	37–38		141	0.81	0.99	0.01	0.01	0.80	3.59	4.46
38	38–39	N5	140	0.80	1.00	0.00	0.00	0.80	2.79	3.49
39	39–40		140	0.80	0.99	0.01	0.01	0.80	1.99	2.49
40	40–41		139	0.79	1.00	0.00	0.00	0.79	1.19	1.50
41	41–42	Adult	139	0.79						

[a] N1 = 1st instar; N2 = 2nd instar; ; N3 = 3rd instar; N4 = 4th instar; N5 = 5th instar

Table 5.3. Survivorship life table for the immature bed bug stages of a pyrethroid-resistant field strain (Kramer, KR).

Age Class, x (days)	Age Interval (days)	Life Stage[a]	Number Alive at Start of Age Interval x, n_x	Fraction Living at Age x, l_x	Fraction Surviving from x to x +1, p_x	Fraction Dying from x to x +1, q_x	Fraction Dying in Interval x to x +1, d_x	Days lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x
0	0–1	Egg	198	1.00	1.00	0.00	0.00	1.00	32.22	32.22
1	1–2		198	1.00	1.00	0.00	0.00	1.00	31.22	31.22
2	2–3		198	1.00	1.00	0.00	0.00	1.00	30.22	30.22
3	3–4		198	1.00	1.00	0.00	0.00	1.00	29.22	29.22
4	4–5		198	1.00	1.00	0.00	0.00	1.00	28.22	28.22
5	5–6		198	1.00	0.88	0.12	0.12	0.94	27.22	27.22
6	6–7	N1	174	0.88	0.98	0.02	0.02	0.87	26.28	29.90
7	7–8		170	0.86	0.98	0.02	0.02	0.85	25.41	29.59
8	8–9		166	0.84	0.99	0.01	0.01	0.84	24.56	29.30
9	9–10		165	0.83	0.99	0.01	0.01	0.83	23.72	28.47
10	10–11		164	0.83	0.98	0.02	0.02	0.82	22.89	27.64
11	11–12		161	0.81	0.96	0.04	0.04	0.80	22.07	27.15
12	12–13		154	0.78	0.97	0.03	0.03	0.77	21.28	27.36
13	13–14		149	0.75	0.99	0.01	0.01	0.75	20.51	27.26
14	14–15	N1	148	0.75	0.98	0.02	0.02	0.74	19.76	26.44
15	15–16		145	0.73	0.99	0.01	0.01	0.73	19.02	25.98
16	16–17		144	0.73	1.00	0.00	0.00	0.73	18.29	25.15
17	17–18		144	0.73	1.00	0.00	0.00	0.73	17.57	24.15
18	18–19		144	0.73	1.00	0.00	0.00	0.73	16.84	23.15
19	19–20		144	0.73	1.00	0.00	0.00	0.73	16.11	22.15
20	20–21		144	0.73	1.00	0.00	0.00	0.73	15.38	21.15
21	21–22		144	0.73	1.00	0.00	0.00	0.73	14.66	20.15

Age Class, x (days)	Age Interval (days)	Life Stage[a]	Number Alive at Start of Age Interval x, n_x	Fraction Living at Age x, l_x	Fraction Surviving from x to x +1, p_x	Fraction Dying from x to x +1, q_x	Fraction Dying in Interval x to x +1, d_x	Days lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x
22	22–23	N3	144	0.73	1.00	0.00	0.00	0.73	13.93	19.15
23	23–24		144	0.73	1.00	0.00	0.00	0.73	13.20	18.15
24	24–25		144	0.73	1.00	0.00	0.00	0.73	12.47	17.15
25	25–26		144	0.73	1.00	0.00	0.00	0.73	11.75	16.15
26	26–27		144	0.73	1.00	0.00	0.00	0.73	11.02	15.15
27	27–28		144	0.73	0.99	0.01	0.01	0.72	10.29	14.15
28	28–29		143	0.72	0.99	0.01	0.01	0.72	9.57	13.25
29	29–30	N4	142	0.72	1.00	0.00	0.00	0.72	8.85	12.34
30	30–31		142	0.72	0.99	0.01	0.01	0.71	8.13	11.34
31	31–32		141	0.71	1.00	0.00	0.00	0.71	7.42	10.41
32	32–33		141	0.71	1.00	0.00	0.00	0.71	6.70	9.41
33	33–34		141	0.71	1.00	0.00	0.00	0.71	5.99	8.41
34	34–35		141	0.71	1.00	0.00	0.00	0.71	5.28	7.41
35	35–36		141	0.71	1.00	0.00	0.00	0.71	4.57	6.41
36	36–37		141	0.71	1.00	0.00	0.00	0.71	3.86	5.41
37	37–38	N5	141	0.71	0.99	0.01	0.01	0.71	3.14	4.41
38	38–39		140	0.71	0.99	0.01	0.01	0.70	2.43	3.44
39	39–40		139	0.70	0.99	0.01	0.01	0.70	1.73	2.46
40	40–41		137	0.69	0.99	0.01	0.01	0.69	1.03	1.49
41	41–42	Adult	136	0.69						

[a] N1 = 1st instar; N2 = 2nd instar; ; N3 = 3rd instar; N4 = 4th instar; N5 = 5th instar

Table 5.4. Survivorship life table for the immature bed bug stages of a pyrethroid-resistant field strain (Richmond, RR).

Age Class, x (days)	Age Interval (days)	Life Stage[a]	Number Alive at Start of Age Interval x, n_x	Fraction Living at Age x, l_x	Fraction Surviving from x to x +1, p_x	Fraction Dying from x to x +1, q_x	Fraction Dying in Interval x to x +1, d_x	Days lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x
0	0–1	Egg	192	1.00	1.00	0.00	0.00	1.00	34.35	34.35
1	1–2		192	1.00	1.00	0.00	0.00	1.00	33.35	33.35
2	2–3		192	1.00	1.00	0.00	0.00	1.00	32.35	32.35
3	3–4		192	1.00	1.00	0.00	0.00	1.00	31.35	31.35
4	4–5		192	1.00	1.00	0.00	0.00	1.00	30.35	30.35
5	5–6		192	1.00	0.99	0.01	0.01	0.99	29.35	29.35
6	6–7		190	0.99	1.00	0.00	0.00	0.99	28.36	28.66
7	7–8	N1	190	0.99	1.00	0.00	0.00	0.99	27.37	27.66
8	8–9		190	0.99	0.98	0.02	0.02	0.98	26.38	26.66
9	9–10		187	0.97	1.00	0.00	0.00	0.97	25.40	26.08
10	10–11		187	0.97	1.00	0.00	0.00	0.97	24.42	25.08
11	11–12	N2	187	0.97	1.00	0.00	0.00	0.97	23.45	24.08
12	12–13		187	0.97	1.00	0.00	0.00	0.97	22.48	23.08
13	13–14		187	0.97	1.00	0.00	0.00	0.97	21.50	22.08
14	14–15		187	0.97	1.00	0.00	0.00	0.97	20.53	21.08
15	15–16		187	0.97	1.00	0.00	0.00	0.97	19.55	20.08
16	16–17		187	0.97	1.00	0.00	0.00	0.97	18.58	19.08
17	17–18	N3	187	0.97	1.00	0.00	0.00	0.97	17.61	18.08
18	18–19		187	0.97	0.99	0.01	0.01	0.97	16.63	17.08
19	19–20		186	0.97	1.00	0.00	0.00	0.97	15.66	16.17

Age Class, x (days)	Age Interval (days)	Life Stage[a]	Number Alive at Start of Age Interval x, n_x	Fraction Living at Age x, l_x	Fraction Surviving from x to x +1, p_x	Fraction Dying from x to x +1, q_x	Fraction Dying in Interval x to x +1, d_x	Days lived in Interval, L_x	Number of Days Lived Beyond Age x, T_x	Expectation of Life, e_x
20	20–21		186	0.97	1.00	0.00	0.00	0.97	14.69	15.17
21	21–22		186	0.97	1.00	0.00	0.00	0.97	13.72	14.17
22	22–23		186	0.97	0.99	0.01	0.01	0.96	12.76	13.17
23	23–24	N4	184	0.96	1.00	0.00	0.00	0.96	11.79	12.30
24	24–25		184	0.96	1.00	0.00	0.00	0.96	10.83	11.30
25	25–26		184	0.96	0.99	0.01	0.01	0.95	9.88	10.30
26	26–27		182	0.95	1.00	0.00	0.00	0.95	8.92	9.41
27	27–28		182	0.95	0.99	0.01	0.01	0.94	7.97	8.41
28	28–29		180	0.94	1.00	0.00	0.00	0.94	7.03	7.50
29	N5	N5	180	0.94	1.00	0.00	0.00	0.94	6.09	6.50
30	30–31		180	0.94	1.00	0.00	0.00	0.94	5.16	5.50
31	31–32		180	0.94	1.00	0.00	0.00	0.94	4.22	4.50
32	32–33		180	0.94	1.00	0.00	0.00	0.94	3.28	3.50
33	33–34		180	0.94	1.00	0.00	0.00	0.94	2.34	2.50
34	34–35		180	0.94	1.00	0.00	0.00	0.94	1.41	1.50
35	35–36	Adult	180	0.94						

[a] N1 = 1st instar; N2 = 2nd instar; ; N3 = 3rd instar; N4 = 4th instar; N5 = 5th instar

Table 5.5. Mean (± 95% Confidence Limits) duration of each life stage (days) for three bed bug strains.

Life Stage	Bed Bug Strain (n = Life Table Cohort Size)		
	Harlan (HS) (n = 175)	Kramer (KR) (n = 198)	Richmond (RR) (n = 192)
Egg	5.41 (5.33 – 5.49) a	5.84 (5.73 – 5.94) b	6.33 (6.24 – 6.41) c
1st instar (N1)	7.82 (7.61 – 8.04) a	7.95 (7.71 – 8.19) a	4.46 (4.35 – 4.57) b
2nd instar (N2)	8.20 (7.93 – 8.48) a	7.80 (7.49 – 8.11) a	6.02 (5.90 – 6.14) b
3rd instar (N3)	8.02 (7.73 – 8.31) a	7.02 (6.68 – 7.36) b	5.62 (5.45 – 5.79) c
4th instar (N4)	7.63 (7.34 – 7.92) a	7.61 (7.28 – 7.95) a	5.66 (5.47 – 5.86) b
5th instar (N5)	3.28 (3.06 – 3.49) a	4.12 (3.88 – 4.37) b	6.73 (6.54 – 6.92) c
Egg – Adult	40.37 (40.18 – 40.56) a	40.35 (40.16 – 40.53) a	34.82 (34.70 – 34.95) b

* Means in each row followed by the same letter are not significantly different ($P > 0.05$)

Table 5.6. Fertility life table for a pyrethroid-resistant field strain (Richmond RR).

Age Class, x (days)	Age Interval (days)	Life Stage	Fraction Living at Age x, l_x	Expectation of Life, e_x	Expected Number of Daughters by Female Alive at Age x, m_x	Reproductive Expectation, $l_x m_x$	$l_x m_x x$
0	0–1	Egg	1.00	142.52			
□	□	□	□	□			
35	35–36	Adult	0.88	124.94	0.00	0.00	0.00
36	36–37	Adult	0.86	126.90	0.00	0.00	0.00
37	37–38	Adult	0.84	128.99	0.00	0.00	0.00
38	38–39	Adult	0.82	131.20	0.00	0.00	0.00
39	39–40	Adult	0.82	130.20	0.05	0.04	1.61
40	40–41	Adult	0.82	129.20	1.73	1.41	57.03
41	41–42	Adult	0.82	128.20	2.03	1.65	68.60
42	42–43	Adult	0.82	127.20	1.23	1.00	42.50
43	43–44	Adult	0.82	126.20	0.88	0.71	31.07
44	44–45	Adult	0.82	125.20	0.23	0.18	8.17
45	45–46	Adult	0.82	124.20	0.08	0.06	2.79
46	46–47	Adult	0.82	123.20	0.03	0.02	0.95
47	47–48	Adult	0.82	122.20	0.00	0.00	0.00
48	48–49	Adult	0.82	121.20	0.00	0.00	0.00
49	49–50	Adult	0.82	120.20	0.00	0.00	0.00
50	50–51	Adult	0.82	119.20	0.00	0.00	0.00
51	51–52	Adult	0.82	118.20	0.00	0.00	0.00
52	52–53	Adult	0.82	117.20	0.00	0.00	0.00
53	53–54	Adult	0.82	116.20	0.50	0.41	21.84
54	54–55	Adult	0.82	115.20	1.18	0.96	52.28
55	55–56	Adult	0.82	114.20	1.60	1.31	72.49

Age Class, x (days)	Age Interval (days)	Life Stage	Fraction Living at Age x, l_x	Expectation of Life, e_x	Expected Number of Daughters by Female Alive at Age x, m_x	Reproductive Expectation, l_xm_x	l_xm_xx
56	56–57	Adult	0.82	113.20	0.85	0.69	39.20
57	57–58	Adult	0.82	112.20	0.13	0.10	5.87
58	58–59	Adult	0.82	111.20	0.60	0.49	28.65
59	59–60	Adult	0.82	110.20	0.00	0.00	0.00
60	60–61	Adult	0.82	109.20	0.00	0.00	0.00
61	61–62	Adult	0.82	108.20	0.00	0.00	0.00
62	62–63	Adult	0.82	107.20	0.00	0.00	0.00
63	63–64	Adult	0.82	106.20	0.00	0.00	0.00
64	64–65	Adult	0.82	105.20	0.00	0.00	0.00
65	65–66	Adult	0.82	104.20	0.00	0.00	0.00
66	66–67	Adult	0.82	103.20	1.83	1.49	99.07
67	67–68	Adult	0.82	102.20	1.75	1.43	96.43
68	68–69	Adult	0.82	101.20	1.88	1.53	104.85
69	69–70	Adult	0.82	100.20	0.68	0.55	38.30
70	70–71	Adult	0.82	99.20	0.40	0.33	23.02
71	71–72	Adult	0.82	98.20	0.05	0.04	2.92
72	72–73	Adult	0.82	97.20	0.00	0.00	0.00
73	73–74	Adult	0.82	96.20	0.00	0.00	0.00
74	74–75	Adult	0.82	95.20	0.00	0.00	0.00
75	75–76	Adult	0.82	94.20	0.00	0.00	0.00
76	76–77	Adult	0.82	93.20	0.10	0.08	6.24
77	77–78	Adult	0.82	92.20	1.65	1.35	104.39
78	78–79	Adult	0.82	91.20	2.08	1.69	132.97
79	79–80	Adult	0.82	90.20	1.03	0.84	66.52
80	80–81	Adult	0.82	89.20	0.65	0.53	42.71
81	81–82	Adult	0.82	88.20	0.25	0.20	16.63
82	82–83	Adult	0.82	87.20	0.00	0.00	0.00
83	83–84	Adult	0.82	86.20	0.00	0.00	0.00

Age Class, x (days)	Age Interval (days)	Life Stage	Fraction Living at Age x, l_x	Expectation of Life, e_x	Expected Number of Daughters by Female Alive at Age x, m_x	Reproductive Expectation, l_xm_x	l_xm_xx
84	84–85	Adult	0.82	85.20	0.00	0.00	0.00
85	85–86	Adult	0.78	88.66	0.00	0.00	0.00
86	86–87	Adult	0.78	87.66	0.00	0.00	0.00
87	87–88	Adult	0.78	86.66	0.00	0.00	0.00
88	88–89	Adult	0.78	85.66	0.00	0.00	0.00
89	89–90	Adult	0.78	84.66	0.61	0.47	42.01
90	90–91	Adult	0.78	83.66	0.63	0.49	44.33
91	91–92	Adult	0.78	82.66	1.13	0.88	80.30
92	92–93	Adult	0.73	86.22	0.46	0.34	31.22
93	93–94	Adult	0.69	90.26	0.60	0.42	38.93
94	94–95	Adult	0.69	89.26	0.18	0.12	11.57
95	95–96	Adult	0.69	88.26	0.09	0.06	5.85
96	96–97	Adult	0.69	87.26	0.00	0.00	0.00
97	97–98	Adult	0.69	86.26	0.00	0.00	0.00
98	98–99	Adult	0.69	85.26	0.00	0.00	0.00
99	99–100	Adult	0.65	89.56	0.00	0.00	0.00
100	100–101	Adult	0.65	88.56	0.00	0.00	0.00
101	101–102	Adult	0.65	87.56	0.00	0.00	0.00
102	102–103	Adult	0.65	86.56	0.00	0.00	0.00
103	102–104	Adult	0.65	85.56	0.00	0.00	0.00
104	104–105	Adult	0.61	90.23	1.45	0.89	92.87
105	105–106	Adult	0.61	89.23	1.43	0.88	92.58
106	106–107	Adult	0.61	88.23	0.97	0.59	63.03
107	107–108	Adult	0.61	87.23	0.67	0.41	43.88
108	108–109	Adult	0.61	86.23	0.30	0.18	19.93
109	109–110	Adult	0.61	85.23	0.00	0.00	0.00
110	110–111	Adult	0.61	84.23	0.00	0.00	0.00
111	111–112	Adult	0.61	83.23	0.00	0.00	0.00

Age Class, x (days)	Age Interval (days)	Life Stage	Fraction Living at Age x, l_x	Expectation of Life, e_x	Expected Number of Daughters by Female Alive at Age x, m_x	Reproductive Expectation, l_xm_x	l_xm_xx
112	112–113	Adult	0.61	82.23	0.00	0.00	0.00
113	113–114	Adult	0.61	81.23	0.00	0.00	0.00
114	114–115	Adult	0.61	80.23	0.00	0.00	0.00
115	115–116	Adult	0.57	84.93	0.07	0.04	4.55
116	116–117	Adult	0.57	83.93	0.32	0.18	21.40
117	117–118	Adult	0.57	82.93	1.29	0.73	86.33
118	118–119	Adult	0.57	81.93	1.64	0.94	111.24
119	119–120	Adult	0.57	80.93	0.93	0.53	63.41
120	120–121	Adult	0.57	79.93	0.57	0.33	39.35
121	121–122	Adult	0.57	78.93	0.18	0.10	12.40
122	122–123	Adult	0.57	77.93	0.21	0.12	15.00
123	123–124	Adult	0.57	76.93	0.00	0.00	0.00
124	124–125	Adult	0.57	75.93	0.00	0.00	0.00
125	125–126	Adult	0.57	74.93	0.00	0.00	0.00
126	126–127	Adult	0.57	73.93	0.00	0.00	0.00
127	127–128	Adult	0.57	72.93	0.00	0.00	0.00
128	128–129	Adult	0.53	77.50	0.00	0.00	0.00
129	129–130	Adult	0.53	76.50	0.00	0.00	0.00
130	130–131	Adult	0.53	75.50	0.69	0.37	47.94
131	131–132	Adult	0.53	74.50	1.08	0.57	75.14
132	132–133	Adult	0.53	73.50	1.46	0.78	102.76
133	133–134	Adult	0.53	72.50	0.81	0.43	57.21
134	134–135	Adult	0.53	71.50	0.38	0.20	27.45
135	135–136	Adult	0.53	70.50	0.54	0.29	38.71
136	136–137	Adult	0.53	69.50	0.00	0.00	0.00
137	137–138	Adult	0.53	68.50	0.00	0.00	0.00
138	138–139	Adult	0.53	67.50	0.00	0.00	0.00
139	139–140	Adult	0.53	66.50	0.00	0.00	0.00

Age Class, x (days)	Age Interval (days)	Life Stage	Fraction Living at Age x, l_x	Expectation of Life, e_x	Expected Number of Daughters by Female Alive at Age x, m_x	Reproductive Expectation, l_xm_x	l_xm_xx
140	140–141	Adult	0.53	65.50	0.00	0.00	0.00
141	141–142	Adult	0.53	64.50	0.00	0.00	0.00
142	142–143	Adult	0.53	63.50	0.00	0.00	0.00
143	143–144	Adult	0.53	62.50	0.00	0.00	0.00
144	144–145	Adult	0.53	61.50	0.00	0.00	0.00
145	145–146	Adult	0.53	60.50	0.00	0.00	0.00
146	146–147	Adult	0.53	59.50	0.00	0.00	0.00
147	147–148	Adult	0.53	58.50	0.15	0.08	12.04
148	148–149	Adult	0.53	57.50	0.65	0.35	51.52
149	149–150	Adult	0.53	56.50	0.65	0.35	51.87
150	150–151	Adult	0.53	55.50	0.62	0.33	49.14
151	151–152	Adult	0.53	54.50	0.81	0.43	64.93
152	152–153	Adult	0.53	53.50	0.50	0.27	40.46
153	153–154	Adult	0.53	52.50	0.35	0.18	28.19
154	154–155	Adult	0.53	51.50	0.00	0.00	0.00
155	155–156	Adult	0.53	50.50	0.00	0.00	0.00
156	156–157	Adult	0.53	49.50	0.00	0.00	0.00
157	157–158	Adult	0.53	48.50	0.00	0.00	0.00
158	158–159	Adult	0.53	47.50	0.00	0.00	0.00
159	159–160	Adult	0.53	46.50	0.00	0.00	0.00
160	160–161	Adult	0.53	45.50	0.00	0.00	0.00
161	161–162	Adult	0.53	44.50	0.00	0.00	0.00
162	162–163	Adult	0.49	47.17	0.00	0.00	0.00
163	163–164	Adult	0.49	46.17	0.00	0.00	0.00
164	164–165	Adult	0.49	45.17	0.00	0.00	0.00
165	165–166	Adult	0.49	44.17	0.33	0.16	27.02
166	166–167	Adult	0.49	43.17	0.46	0.22	37.38
167	167–168	Adult	0.49	42.17	0.50	0.24	41.02

Age Class, x (days)	Age Interval (days)	Life Stage	Fraction Living at Age x, l_x	Expectation of Life, e_x	Expected Number of Daughters by Female Alive at Age x, m_x	Reproductive Expectation, l_xm_x	l_xm_xx
168	168–169	Adult	0.49	41.17	0.33	0.16	27.51
169	169–170	Adult	0.49	40.17	0.17	0.08	13.84
170	170–171	Adult	0.49	39.17	0.00	0.00	0.00
171	171–172	Adult	0.49	38.17	0.00	0.00	0.00
172	172–173	Adult	0.49	37.17	0.00	0.00	0.00
173	173–174	Adult	0.49	36.17	0.00	0.00	0.00
174	174–175	Adult	0.49	35.17	0.00	0.00	0.00
175	175–176	Adult	0.49	34.17	0.00	0.00	0.00
176	176–177	Adult	0.49	33.17	0.00	0.00	0.00
177	177–178	Adult	0.49	32.17	0.00	0.00	0.00
178	178–179	Adult	0.49	31.17	0.00	0.00	0.00
179	179–180	Adult	0.45	32.95	0.00	0.00	0.00
180	180–181	Adult	0.45	31.95	0.00	0.00	0.00
181	181–182	Adult	0.45	30.95	0.00	0.00	0.00
182	182–183	Adult	0.45	29.95	0.00	0.00	0.00
183	183–184	Adult	0.45	28.95	0.00	0.00	0.00
184	184–185	Adult	0.45	27.95	0.50	0.22	41.42
185	185–186	Adult	0.41	29.70	0.62	0.25	46.87
186	186–187	Adult	0.41	28.70	0.65	0.27	49.48
187	187–188	Adult	0.41	27.70	0.45	0.18	34.44
188	188–189	Adult	0.41	26.70	0.25	0.10	19.23
189	189–190	Adult	0.41	25.70	0.00	0.00	0.00
190	190–191	Adult	0.41	24.70	0.00	0.00	0.00
191	191–192	Adult	0.41	23.70	0.00	0.00	0.00
192	192–193	Adult	0.41	22.70	0.00	0.00	0.00
193	193–194	Adult	0.41	21.70	0.00	0.00	0.00
194	194–195	Adult	0.41	20.70	0.00	0.00	0.00
195	195–196	Adult	0.37	21.94	0.00	0.00	0.00

Age Class, x (days)	Age Interval (days)	Life Stage	Fraction Living at Age x, l_x	Expectation of Life, e_x	Expected Number of Daughters by Female Alive at Age x, m_x	Reproductive Expectation, l_xm_x	l_xm_xx
196	196–197	Adult	0.37	20.94	0.00	0.00	0.00
197	197–198	Adult	0.37	19.94	0.00	0.00	0.00
198	198–199	Adult	0.37	18.94	0.33	0.12	24.31
199	199–200	Adult	0.33	20.25	0.29	0.10	19.16
200	200–201	Adult	0.33	19.25	0.31	0.10	20.46
201	201–202	Adult	0.33	18.25	0.25	0.08	16.45
202	202–203	Adult	0.33	17.25	0.06	0.02	4.13
203	203–204	Adult	0.33	16.25	0.00	0.00	0.00
204	204–205	Adult	0.33	15.25	0.00	0.00	0.00
205	205–206	Adult	0.33	14.25	0.00	0.00	0.00
206	206–207	Adult	0.33	13.25	0.00	0.00	0.00
207	207–208	Adult	0.33	12.25	0.00	0.00	0.00
208	208–209	Adult	0.33	11.25	0.00	0.00	0.00
209	209–210	Adult	0.29	11.79	0.00	0.00	0.00
210	210–211	Adult	0.29	10.79	0.00	0.00	0.00
211	211–212	Adult	0.29	9.79	0.00	0.00	0.00
212	121–213	Adult	0.24	10.33	0.00	0.00	0.00
213	213–214	Adult	0.24	9.33	0.00	0.00	0.00
214	214–215	Adult	0.24	8.33	0.00	0.00	0.00
215	215–216	Adult	0.20	8.90	0.00	0.00	0.00
216	216–217	Adult	0.20	7.90	0.00	0.00	0.00
217	217–218	Adult	0.20	6.90	0.00	0.00	0.00
218	218–219	Adult	0.16	7.50	0.00	0.00	0.00
219	219–220	Adult	0.16	6.50	0.25	0.04	8.96
220	220–221	Adult	0.12	7.50	0.43	0.05	11.57
221	221–222	Adult	0.12	6.50	0.33	0.04	9.04
222	222–223	Adult	0.12	5.50	0.33	0.04	9.08
223	223–224	Adult	0.12	4.50	0.00	0.00	0.00

Age Class, x (days)	Age Interval (days)	Life Stage	Fraction Living at Age x, l_x	Expectation of Life, e_x	Expected Number of Daughters by Female Alive at Age x, m_x	Reproductive Expectation, $l_x m_x$	$l_x m_x x$
224	224–225	Adult	0.12	3.50	0.17	0.02	4.58
225	225–226	Adult	0.12	2.50	0.33	0.04	9.20
226	226–227	Adult	0.12	1.50	0.50	0.06	13.87
227	227–228	Adult	0.12	0.50	0.67	0.08	18.57
228	228–229	Adult	0.00		0.00	0.00	0.00

Table 5.7. Population parameters for the Richmond (RR) strain of *C. lectularius* at 26 ºC and 68.9% R.H

Population Parameter	Calculation	Value
Gross Reproductive Rate	$\sum m_x$	54.34
Net Replacement rate (R_0)	$\sum l_x m_x$	35.12
Mean generation time (G)	$\frac{\sum l_x m_x x}{\sum l_x m_x} = \frac{\sum l_x m_x x}{R_0}$	92.31
Intrinsic rate of increase (approximate)	$\frac{\log_e R_0}{G}$	0.0386
Intrinsic rate of increase (precise)	$\sum e^{-rx} l_x m_x = 1.0$	0.0539

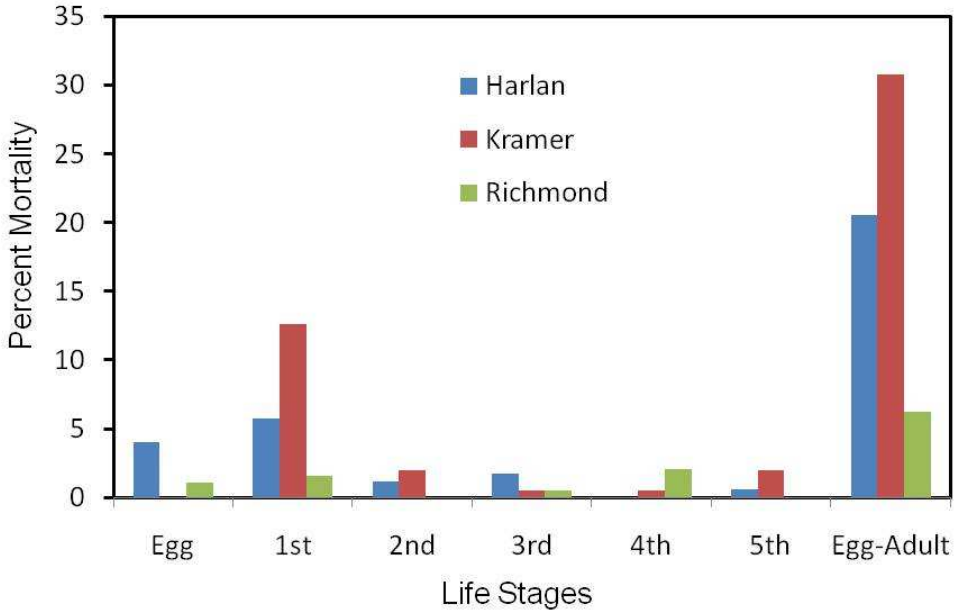

Figure 5.1. Percent mortality within each life stage for Harlan (HS susceptible), Kramer (KR resistant), and Richmond (RR resistant) bed bug strains, *Cimex lectularius.*

Chapter 6: Stage-Based Model for Bed Bug, C. *lectularius* L.

Introduction

Successful pest control relies on having a good understanding of the biology, ecology, and population dynamics of a pest (Pimentel 1966). In a previous study, we developed life tables for the common bed bug to gather information to fill the gap in our knowledge of the biology of the insect. Before the development of DDT, bed bugs were a major urban pest around the world. With the development of DDT during World War II came a reduction in the bed bug populations. However, after 1972, when DDT was discontinued due to its potential toxicity to the environment and human health (Potter 2006) a resurgence of bed bug population outbreaks occurred. The absence of outbreak populations of bed bug for almost 40 years was marked by the absence of research on the insect (Harlan 2006), so much so that the most comprehensive account of the ecology of the insect was complied almost 70 years ago (e.g., Johnson 1941).

There are many theories to explain the increase in bed bug infestations in the U.S. These include, an increase in international travel (Boase 2001), immigration from other countries (Cooper 2006, Potter 2007), seasonal human migration, such as recreational travel (Kells 2006), lack of education, misidentification of bed bugs (Cooper 2006), and resistance to insecticides used for bed bug control (Potter 2006). Several recent studies have in fact documented resistance to pyrethroid insecticide in *C. lectularius* (Moore and Miller 2006, Romero et al. 2007, Kyong et al. 2008) and in *C. hemipterus* (Karunaratne et al. 2007).

Reinhhardt (2007) stressed the need for ecological and biological studies to understand the life history of bed bug populations in the modern indoor environments within the U.S. To this end, life tables were developed in a previous study (see Life Table chapter) to provide us with updated information on the biology of the pest. The data contained within a life tables can

be used to develop a deeper understanding of the ecology and dynamics of an insect. One way to do this is to use the life tables as a source of information for the development of population models (Harcourt 1969, Southwood 1978). According to Harcourt (1969), life tables help to "identify and evaluate the relative importance of the independent variables" causing mortality, which can then be used to develop models for studying the behavior of the population under certain conditions. Good examples of how life table data might be combined with population modeling to study aspects of the insect pest dynamics can be found in Carey (1982) for the Mediterranean fruit fly and Choi and Ryoo (2003) for *Paronychiurus kimi*.

Although the early models developed for use in IPM were mostly simulations models (Coulson and Saunders 1987, Bommarco 2001) simpler modeling such as population projection-matrix models (Shea and Kelly 1998, Bommarco 2001) have proven to be as effective. Projections matrix models can incorporate in simple fashion age-or stage-specific biological information on development, mortality, and fertility of individuals in the population with information of biotic (e.g., natural enemies) and abiotic (e.g., temperature) factors that might affect the population (Caswell 2001). These models can be analyzed analytically and/or used as simulation models for projecting the population through time. As such, projection matrix models represent a middle ground in population modeling approaches between the predictability of statistical (regression) models and the complexity of simulation models (Yu et al. 1992).

The purpose of the present study was to develop stage-classified projection matrix models to gain insights into the population characteristics and dynamics of two bed bug strains. The first strain is one that has been shown to be highly susceptible to pyrethroid insecticide and the other strain is one that is highly resistant to the same insecticides. We used data from life tables to develop the models and to determine the population characteristics such as the intrinsic

rate of increase, stable age distribution, reproductive values, and sensitivity of the population growth rate to changes in vital rates. In addition, for comparison, we also developed and analyzed a projection matrix model for another parasitic insect of human, the human louse, *Pediculus humanus* L., using life table data presented in Evans and Smith (1952).

Materials and Methods

Bed bugs strains

A stage-based population projection model was developed for each of two strains of the common bed bug using data from life tables on the insect (see Chapter 5). For the first strain, Harlan, the projection model was developed based on data from an abridged (egg to 5th instar) morality life table constructed for a laboratory population that was developed from individuals obtained in February 2005 from a laboratory population maintained by Dr. Harold Harlan (National Pest Management Association, Fairfax, VA). The projection model for the second strain, Richmond (RR), was developed using information from a complete (mortality and fertility) life table constructed for a field population that was developed using individuals collected in September 2008 from a multi-room adult home residence in Richmond, VA. The susceptibility of both strains of bed bugs was tested to determine their LT_{50} values with respect to pyrethroid insecticides. Table 6.1 shows that the Harlan (HS) strain was highly susceptible to the pyrethroids tested, while high levels of resistance were observed for the Richmond (RR) strain.

Stage-based projection model

The first step in developing the population projection matrix model for each bed bug strain to construct a Coates life-cycle graph (Caswell 2001) for the seven-stage life cycle of the insect (Fig. 6.1). In Fig. 6.1, *E* represents the egg stage, *N1* to *N5* are the five larval stages, and *Ad* is the adult stage. The probability of individuals in the *i*th stage surviving (S_i) and developing (D_i) to the next stage is $G_i = S_iD_i$; the probability of individuals in the *i*th stage surviving (S_i) and not developing ($1 - D_i$) to the next stage is $P_i = S_i(1 - D_i)$; F_{Ad} is the fecundity of adult females.

The life-cycle graph in Fig. 6.1 can be represented mathematically as,

$$\begin{aligned}
E_{t+1} &= P_1 E_t + F_A Ad \\
N1_{t+1} &= G_1 E_t + P_2 N1_t \\
N2_{t+1} &= G_2 N1_t + P_3 N2_t \\
N3_{t+1} &= G_3 N2_t + P_4 N3_t \\
N4_{t+1} &= G_4 N3_t + P_5 N4_t \\
N5_{t+1} &= G_5 N4_t + P_6 N5_t \\
Ad_{t+1} &= G_6 N5_t + P_7 Ad_t
\end{aligned} \tag{6.1}$$

where the stage abundances at time $t+1$ are derived from knowledge of the stage abundances at time t. In matrix form, equation (6.1) becomes,

$$\begin{bmatrix} E \\ N1 \\ N2 \\ N3 \\ N4 \\ N5 \\ Ad \end{bmatrix}_{t+1} = \begin{bmatrix} P_1 & 0 & 0 & 0 & 0 & 0 & F_{Ad} \\ G_1 & P_2 & 0 & 0 & 0 & 0 & 0 \\ 0 & G_2 & P_3 & 0 & 0 & 0 & 0 \\ 0 & 0 & G_3 & P_4 & 0 & 0 & 0 \\ 0 & 0 & 0 & G_4 & P_5 & 0 & 0 \\ 0 & 0 & 0 & 0 & G_5 & P_6 & 0 \\ 0 & 0 & 0 & 0 & 0 & G_6 & P_7 \end{bmatrix} \begin{bmatrix} E \\ N1 \\ N2 \\ N3 \\ N4 \\ N5 \\ Ad \end{bmatrix}_t \tag{6.2}$$

which in turn can be represented simply as,

$$\boldsymbol{n}_{t+1} = \boldsymbol{A}\boldsymbol{n}_t \tag{6.3}$$

where $\boldsymbol{n}_t$ and $\boldsymbol{n}_{t+1}$ are vectors of the abundances of the seven life stages at time t and $t+1$, respectively; $\boldsymbol{A}$ is the population projection (transition) matrix, which for a stage-classified population is often referred to as the Leftkovich matrix (Lefkovich 1965). Our main interest in this study is the projection matrix, $\boldsymbol{A}$.

The next step in developing the population projection model for the bed bug is to conduct demographic studies on the insect (see Life Table chapter). The information in the life table for

each of the two strains was used to parameterize the elements G_i, P_i, and F_{Ad} of the respective projection matrix, $\boldsymbol{A}$, using methods described in Walls et al. (1991), Carey (1993), Caswell (2001), and Morris and Doak (2001). Once the elements of $\boldsymbol{A}$ had been parameterized, the matrix can be analyzed and/or projected through time to assess the growth and viability characteristics of the population of each bed bug strain.

From analysis of $\boldsymbol{A}$ we obtained the dominant eigenvalue, λ_m, which represents the maximal population growth rate and is equal to e^r, where r is the intrinsic rate of increase of the population in the equation,

$$N_t = N_0 e^{rt} \tag{6.4}$$

When λ_m is 1, $e^r = 0$ and population is considered to be stable.

Analysis of the projection matrix, $\boldsymbol{A}$, also generates a right eigenvector, $\boldsymbol{w}_m$, such that

$$\boldsymbol{A}\boldsymbol{w}_m = \lambda_m \boldsymbol{w}_m \tag{6.5}$$

$\boldsymbol{w}_m$ represents the stable stage structure of the population or the proportion of individuals in each stage class to which the population would converge in a constant environment (Crouse et al. 1987). Also generated is the corresponding left eigenvector, $\boldsymbol{v}$`, calculated by,

$$\boldsymbol{v}`\boldsymbol{A} = \lambda_m \boldsymbol{v}` \tag{6.6}$$

represents the reproductive values or the average contribution that each of the life stages makes to future generations (Crouse et al. 1987).

We can measure the distance between the stable stage distributions of two populations. Caswell (2001) describes several methods for measuring this distance. One method is Keyfitz's Δ, which ranges from 0–1, with 0 indicating that two stage distributions are identical.

Finally, the projection matrix, $\boldsymbol{A}$, was subjected to sensitivity analysis to test the sensitivity of the population growth rate λ_m (and the corresponding *r*-value) to variations in the growth, survival, and fecundity parameters. The results of these analyses are reported as elasticity, which is a measure of proportional sensitivity representing the proportional change in λ_m resulting from a proportional change in one of the life cycle parameters in $\boldsymbol{A}$ (i.e. G_i, P_i, or F_{Ad}) or (Crouse et al. 1987, Caswell 2001). Because the elasticities of the matrix elements sum to 1, the relative contributions of each of the elements of the projection matrix to λ_m can be compared directly. G_i, and P_i in the projection matrix are derived parameters based on the relationship between stage survival (S_i) and stage development (D_i). Because of this, it is also useful to determine the sensitivity of λ_m to these low level vital rates (i.e., to S_i and D_i). We therefore, extended the sensitivity analysis for this purpose.

Demographic Parameters for the bed bug matrix models

The G_i, and P_i values of the projection matrix, $\boldsymbol{A}$, were estimated for the HS and RR strains of the common bed bug using data on survival and stage duration (development) obtained from life tables for each of the strains (see Life Table chapter). The value of F_{Ad} for the RR strain was obtained from analysis of the fertility life table, while F_{Ad} for the HS strain was estimated from a separate study of female fecundity. Also, F_{Ad} represented female eggs only and was estimated assuming a 1:1 sex ratio. All of the parameters of $\boldsymbol{A}$ were estimated based on a projection interval of 1 day. All analyses of the projection matrices were carried out using code written in MATLAB (Mathworks Inc.).

Projection model for the human louse, Pediculus humanus L.

The study by Evans and Smith (1952) on the human louse is a classic example of a life table for a parasitic insect of humans. We developed a projection model for the human louse using the life table data of Evans and Smith (1952) and analyzed the matrix in a manner similar to that described above for the projection matrices of the bed bug strains. In so doing, we were able to directly compare the results of analysis of the population projection matrices for two parasitic species of insects of human, i.e., the bed bug and human louse.

The projection model developed for the human louse is,

$$\begin{bmatrix} E \\ L1 \\ L2 \\ L3 \\ Ad \end{bmatrix}_{t+1} = \begin{bmatrix} P_1 & 0 & 0 & 0 & F_{Ad} \\ G_1 & P_2 & 0 & 0 & 0 \\ 0 & G_2 & P_3 & 0 & 0 \\ 0 & 0 & G_3 & P_4 & 0 \\ 0 & 0 & 0 & G_4 & P_5 \end{bmatrix} \begin{bmatrix} E \\ L1 \\ L2 \\ L3 \\ Ad \end{bmatrix}_t \quad \textbf{(6.7)}$$

where E represents the egg stage, $L1$ to $L3$, the three larval stages, and Ad the adult stage. P_i, G_i, and F_{Ad} are as described for the bed bug.

Results

The estimates of stage-specific survival and development probabilities, and fecundity for the two bed bug strains (HS and RR) are shown in Table 6.2. The stage class projection matrices, **A**, for the HS and RR strains based on the parameter values in Table 6.2 are shown in Tables 6.3 and 6.4, respectively. Similarly, the parameterized projection matrix, **A**, for the human louse, which was derived from the life table data of Evans and Smith (1952), is presented in Table 6.5.

The dominant eigenvalue and intrinsic rate of increase estimated from the projection matrices for the HS and RR bed bug strains were $\lambda_m = 1.0485$ and $r = 0.0474$, and $\lambda_m = 1.0553$ and $r = 0.0538$, respectively; the dominant eigenvalue and intrinsic rate of increase derived from the projection matrix for the human louse was $\lambda_m = 1.1142$ and $r = 0.1082$.

The stable stage distributions, $\boldsymbol{w}$, and reproductive values, $\boldsymbol{v}$, for the HS and RR bed bug strains are given in Table 6.6. The stable stage distributions of both strains were dominated by the egg stage, which comprised >30% of the populations. Approximately 59% of individuals in the stable stage population for both the HS and RR bed bug strains are in the nymphal stages (N1 to N5). Fig. 6.2 shows the differences between the stable stage distributions of the two strains of bed bug with respect to each of the life stages. The egg and N1 stages represent 1% and 8% more of the population, respectively, in the HS strain compared with the RR strain. The RR strain had a higher proportion of its stable age distribution in the N2 to adult life stages compared with the HS strain, with the largest difference occurring in the N5 stage (4%). Despite these differences, there was no significant difference between the stable stage distribution of the two strains ($\chi 2 = 9.1188$, df = 6, $P = 0.167$). This result was confirmed by the Keyfitz's Δ value of 0.09, which is close to zero.

The stable stage distribution from analysis of the projection matrix for the human louse is presented in Table 6.7. Similar to the bed bug populations, the stable population of the human louse is dominated by the egg stage, followed by the immature stages, and the adult stage. Table 6.7 also shows the stable stage distribution calculated by Evans and Smith (1952) directly from the life table. Although the stage-specific values of the stable distribution from the life table differs from those derived from the projection matrix, the two distributions are not statistically different ($\chi 2 = 3.7064$, df = 4, $P = 0.4472$). Again, this result is confirmed by the Keyfitz's Δ value of 0.09, which is close to zero.

The reproductive values, $\boldsymbol{v}$, for the HS and RR strains of bedbug are also shown in Table 6.6. The reproductive values for both bed bug strains increased from egg to the adult stage. The reproductive contributions of life stages other than egg were higher for the HS strain than for the RR strain. Specifically, the contributions of the N1, N2, N3, N4, N5, and adult stages were ≈5, 35, 43, 51, 49, and 40% greater for the HS, respectively, compared with the contributions of these life stages in the RR strain. A similar trend of increasing reproductive values from egg to adult stage was observed for the human louse (Table 6.7). Interestingly, the reproductive values for the egg stage for both bed bug strains and the human louse were 1.0.

The trends in elasticities of λ_m to changes in F_{Ad}, P_i, and G_i for the two bed bug strains are shown in Fig. 6.3. For both strains, λ_m was least sensitive to changes in adult fecundity (F_{Ad}). The sensitivity of λ_m to changes in the probability of surviving and developing, G_i, was similar for the two strains. λ_m , however, appeared to be most sensitive to changes in the probability of survival while remaining in the stage, P_i. Overall, for both strains, changes in P_i for the adult stage are expected to have the greatest impact on λ_m compared with changes in P_i

for the other life stages. For the HS strain, however, the least impact on λ_m from changes in P_i is expected to come for the 5st instar (N5), whereas the least impact on λ_m from changes in P_i for the RR strain is expected to come from the 1st instar (N1).

The patterns of sensitivity of λ_m to changes in F_{Ad}, P_i, and G_i for the human louse (Fig. 6.4) were similar to those observed for the bed bug strains with changes in P_i having a greater impact on population growth compared with changes in F_{Ad} and G_i . Again, similar to the bed bug, changes in the P_i of the adult stage are expected to have the greatest impact on population growth.

Because P_i, and G_i are derived from low level vital rates of stage-specific survival probabilities (S_i) and stage-specific development (D_i), the sensitivity of λ_m to changes in these vital rates was also examined. The results of the analyses are shown in Fig. 6.5 for the bed bug strain and in Fig. 6.6 for the human louse. Not surprisingly, the sensitivity of λ_m to changes in survival probability of the adult stage was highest for both bed bug strains. Other than this, the sensitivity of λ_m to changes in survival probability for all other stages was generally similar for all other stages, expect for the N5 stage for which the sensitivity was lower for the HS strain compared with the RR strain. With respect to development, the sensitivity of λ_m to changes in stage development probabilities was generally higher for the N1, N2, and N3 stages, and lower for the N4 and N5 stages of the HS strain compared with the RR strain.

The patterns of sensitivities for the low level vital rates for the human louse (Fig. 6.6) were similar to those obtained for the bed bug. Again, the sensitivity of λ_m to changes in survival probability of the adult stage was the highest. The sensitivity of λ_m to changes in stage

development for the human louse (Fig. 6.6) indicate that population growth is highly sensitive to changes in egg development.

Discussion

The *r*-value calculated from the projection matrix for the RR strain ($r = 0.0538$) is similar to the value (0.0539) calculated directly from life table data for the strain (see Chapter 5). Also, the *r*-value from analysis of the projection matrix for the human louse ($r = 0.108$) is similar to the value calculated directly from a life table ($r = 0.111$) by Evans and Smith (1952). These two results suggest that both the seven-stage projection matrix for the RR bedbug strain and the five-stage projection matrix for the human louse were adequate representations of the life table data used to parameterize the respective projection matrices. By extension, we might also assume that the projection matrix for the HS bed bug strain adequately represented the seven-stage life cycle for that insect.

The growth rate for the HS bed bug strain ($\lambda_m = 1.0485$; $r = 0.0474$) was slightly lower than that for the RR strain ($\lambda_m = 1.0553$; $r = 0.0538$). The positive growth rates for both strains, however, indicate that the populations are projected to grow if the conditions under which the studies were conducted were held constant. Indeed, we can use the *r*-value for the species to estimate the doubling time ($D = \ln 2 / r$) or the number of days it would take the population to double in size (Russo et al. 2004) under the study conditions. The doubling times for the HS and RR bed bug strains are estimated to be ≈14.6 and 12.9 days, respectively. The doubling time for the human louse based on the *r*-value from the projection matrix is estimated at 6.42 days, a value which is similar to that ($D = 6.24$ days) estimated by Evans and Smith (1952) based on the life table data. The doubling times indicate that under similar environmental conditions it would take a population of the HS bed bug strain ≈2 days longer to double in size compared with the RR strain.

One might speculate that the difference in fitness, as measured by fecundity, developmental time, and life-span (Wood and Bishop 1981) of the two bed bug strains is related to the difference in insecticide susceptibility between the two strains. Indeed, fitness has generally been shown to be reduced as a consequence of insecticide resistance in several insect species (Grayson 1954, Roush and Plapp 1982, Uyenomaya 1986, Krafsur et al. 1993, Carriere 1994, Groeters et al. 1994, Hollinsgsworth 1997, Kumar et al. 2009, Scott 1997, Roy et al 2010). There is only one case that we know of, that of the apterous aphid, *Myzus persicae* where the fitness parameters for a resistance strain were the same or higher than those of the susceptible strain (Banks and Needham 1970). We have seen that the fecundity and developmental time for the RR strain are lower than for the HS strain, a fact that fits well with previous observations. The RR strain, however, may compensate for the lower life history parameters with a higher population growth rate.

Positive *r*-values are not uncommon for insect and mite species. As mentioned earlier, Evans and Smith (1952) calculated an *r*-value of 0.111/day for the louse, and also listed *r*-values of 0.101 and 0.109 for the flour beetle, *Tribolium casttaneum* and rice weevil, *Calandra oryzae*, respectively. Taylor (1979) compiled a list of the life history parameters for 27 species, which included several agricultural insect pests and natural enemies, and three mite species. The *r*-values for all species were positive and ranged between 0.04 and 0.31. The two species in the order Hemiptera, *Lygus hesperus* (Miridae) and *Dysdercus fasciarus* (Pyrrhocoridae), had *r*-values of 0.0742 and 0.0603, respectively. The *r*-values reported for insects and mites in more recent studies (e.g., Hansen et al. 1999, Russo et al. 2004, Gabre et al. 2005, Afrane et al. 2007, Babin et al. 2008) also fall well within the range reported by Taylor (1979).

In discussing the relevance of the *r*-value, Evans and Smith (1952) reminded us that *r*, the intrinsic rate of increase of a population, is conditioned by the specifics of the environment (Birch 1948) and that for each species there is an expected upper limit to *r*. They noted that the laboratory environment under which most life table studies are conducted should provide conditions that are approximately optimal for the species thus allowing them to realize the maximal or nearly maximal rates of increase. Further, Evans and Smith (1952) argued that under natural conditions the *r*-value for a species should approach zero (i.e., λ will approach 1.0) indicating the population is stable and in balance with the conditions of its preferred environment. As such, the distance of the maximal *r*-value (obtained under optimal conditions such as in the laboratory) from zero can provide a measure of the departure of the natural conditions of the species from the optimum. Based on this reasoning, we can speculate that the conditions of the laboratory environment under which the bed bug strains were studied were closer to those of their natural environment than were the conditions under which Evans and Smith (1952) studied the human louse. We can also assume that because of the slightly lower *r*-value that the conditions of the study in the laboratory were slightly closer to the "natural" or optimal environment of the HS strain than it was for the RR strain. This is not surprising since the HS strain has been reared under the laboratory conditions for more than 37 years.

The analyses indicated that there was no significant difference between the stable stage distribution of the HS and RR strains of bed bug, and that the stable stage distributions of both strains were dominated by the egg stage. Both the analysis of the life table (Evans and Smith 1952) and the projection matrix in this study show that the stable stage distribution of the human louse is also dominated by the egg stage. A high percentage of eggs and immature stages have been observed in the stable age distributions for insect and mite species. Birch (1948), for

example, observed a stable age distribution of 95.5% immature stages and 4.5 % adult stage for the rice weevil, *Calandra oryzae*; Carey (1983) observed a the stage age distribution for the tetranychid mite of 63.6% eggs, 27.6% immature, and 8.8% adults. Andrewartha and Birch (1982) discussed the importance of the egg or immature-weighted age distributions with respect to population sampling and noted that depending on the sampling method used the numbers of adults obtained can lead to misleading conclusions of the true size of the population. They emphasized that sampling methods are required that also account for the immature stages in the population.

The results of the sensitivity analysis on the higher (F_{Ad}, P_i, and G_i) and low level vital rates (S_i and D_i) for the two bed bug strains (Figs. 6.3 and 6.5) show that the population growth rates are highly sensitive to changes in adult survival probabilities. A similar trend in elasticity was observed for the human louse with the population growth rate being highly sensitive to survivorship of adults (Figs, 6.4 and 6.6). Similar patterns of sensitivity of population growth rate to changes in adult survival were observed by Bommarco (2001) when studying the influence of temperature on the population dynamics of several arthropod pests. These results suggest that any strategy that reduces the survivorship of adults will likely also greatly reduce the growth potential of both the bed bug and human louse populations.

Interestingly, the sensitivity of population growth rate to changes in survivorship of the N5 stage is lower for the HS strain compared with the RR strain. In addition, changes in survivorship of the N5 stage appear to have the least potential impact on HS population growth compared with all other changes (Fig. 6.5). The reason for this is unclear but may be related to development duration, which is a measure of the period of exposure and vulnerability to the environment for the stage. The mean development duration of the N5 stage for the HS strain

(3.3 days) is half that of the RR strain (6.7 days) (see Life Table chapter). This implies that the N5 stage of the HS strain is exposed to, and therefore is less vulnerable to the environment for a shorter time than the N5 stage of the RR strain. It seems plausible for there to be a strong positive correlation between stage exposure time and elasticity of stage survival. Indeed, we found this to be true for the immature stages of both the HS ($\rho = 0.92$) and RR ($\rho = 0.98$) strains. It is not surprising then that the elasticity of survival for the adult stage, which has the longest exposure time, is highest among all life stages. This emphasizes that control efforts for the bed bug would benefit by targeting the adult stages because of its long period of exposure. It is for this very reason that Legaspi and Legaspi (2007) suggested that the egg stage of the cactus moth, *Cactoblastis cactorum* (Berg) should be targeted for control because it is the most exposed stage with a relatively long duration.

Also interesting is the similarity among the life stage sensitivity patterns for the two bed bug strains (Figs. 6.3 and 6.5) and the human louse (Figs, 6.4 and 6.6). For both insect species the elasticities of stage survival generally increased from egg to adult stage. Brommarco (2001) found a similar pattern of elasticity Mediterranean fruit fly, *Ceratitis capitata*, which suggests that the pattern may be common for insect species. Indeed, the data used to derive the projection matrices for the bed bug strains and the human louse were obtained from studies of populations of these insects, under laboratory conditions. As pointed out by Evans and Smith (1952), laboratory conditions are conducive for obtaining maximal longevity and fecundity and may be considered optimal for the species.

It is clear from these analyses that the key to the reduction of the populations of bed bugs lies with the reduction of survival of the adults. However, there are several reasons why management of the insect has so far been ineffective. The reasons include the cryptic behavior of

the insect, which reduces contact with insecticides, the lack of residual activity of the applied insecticides, lack of economical, safe, and effective alternatives, and lack of efficient sampling methods for the insect.

Table 6.1. Time to mortality of adult bed bugs (50% male: 50% female) confined on hardboard panels treated with residual insecticides. Field strains and laboratory susceptible strain (Harlan) were run in the same day for comparison

Bed Bug Strain	Treatment[a]	LT_{50} (% h)	95% CLs	Resistance ratios	Source
Harlan (HS)	Suspend SC	0.82	0.72–0.94	---	Unpublished data (February 2009)
	Dragnet	1.48	1.23–1.78	---	
Richmond (RR)	Suspend SC	320.24	304.40–338.18	390.5	Unpublished data (February 2009)
	Dragnet	>432.00	---	291.7	

[a] Active ingredient in Suspend SC is Deltamethrin (0.06%); active ingredient in Dragnet is Permethrin (0.05%)

Table 6.2. Estimated parameter values of the stage-based projection matrices for two bed bug strains.

Strain	Life Stage	D_i	S_i	P_i	G_i	F_A
Harland (HS)	Egg	0.1848	0.9600	0.7826	0.1774	
	N1	0.1278	0.9367	0.8170	0.1197	
	N2	0.1219	0.9865	0.8663	0.1202	
	N3	0.1247	0.9792	0.8571	0.1221	
	N4	0.1311	1.0000	0.8689	0.1311	
	N5	0.3050	0.9929	0.6901	0.3028	
	Adult	0.0000	0.9558	0.9558	0.0000	1.035
Richmond (RR)	Egg	0.1581	0.9896	0.8332	0.1564	
	N1	0.2241	0.9842	0.7637	0.2206	
	N2	0.1661	1.0000	0.8339	0.1661	
	N3	0.1779	0.9947	0.8177	0.1769	
	N4	0.1766	0.9783	0.8055	0.1728	
	N5	0.1486	1.0000	0.8514	0.1486	
	Adult	0.0000	0.9558	0.9558	0.0000	0.648

Table 6.3. Stage-class projection matrix, A, for the Harlan (HS) bed bug strain based on the estimated parameter values listed in Table 6.2.

0.7826	0	0	0	0	0	1.0350
0.1774	0.8170	0	0	0	0	0
0	0.1197	0.8663	0	0	0	0
0	0	0.1202	0.8571	0	0	0
0	0	0	0.1221	0.8689	0	0
0	0	0	0	0.1311	0.6901	0
0	0	0	0	0	0.3028	0.9558

Table 6.4. Stage-class projection matrix, A, for the Richmond (RR) bed bug strain based on the estimated parameter values listed in Table 6.2.

0.8332	0	0	0	0	0	0.6480
0.1564	0.7637	0	0	0	0	0
0	0.2206	0.8339	0	0	0	0
0	0	0.1661	0.8177	0	0	0
0	0	0	0.1769	0.8055	0	0
0	0	0	0	0.1728	0.8514	0
0	0	0	0	0	0.1486	0.9558

Table 6.5. Stage-class projection matrix, *A*, for the human louse. *Pediculus humanus* based on the life table data developed by Evans and Smith (1952).

0.7676	0	0	0	2.5300
0.1094	0.7743	0	0	0
0	0.1831	0.6827	0	0
0	0	0.2845	0.7327	0
0	0	0	0.2304	0.9430

Table 6.6. Stable stage distribution (w_m) and reproductive values (v`) for the projection matrices in Tables 6.3 and 6.4 for the Harlan and Richmond bed bug strains, respectively.

Strain	Life Stage	Stable stage distribution, % (Right eigenvector)	Reproductive values (Left eigenvector)
Harland (HS)	Egg	31.88	1.00
	1st instar (N1)	24.43	1.50
	2nd instar (N2)	16.05	2.90
	3rd instar (N3)	10.08	4.39
	4th instar (N4)	6.86	6.89
	5th instar (N5)	2.51	9.43
	Adult	8.19	11.17
Richmond (RR)	Egg	30.58	1.00
	1st instar (N1)	16.41	1.42
	2nd instar (N2)	16.35	1.88
	3rd instar (N3)	11.43	2.50
	4th instar (N4)	8.10	3.36
	5th instar (N5)	6.87	4.85
	Adult	10.26	6.66

Table 6.7. Stable stage distribution and reproductive values for the projection matrix for the human louse in Table 6.5. Also, shown is the stable stage distribution calculated by Evans and Smith (1952) from life table analysis.

Life Stage	Stable stage distribution, % (Right eigenvector)	Stable stage distribution Evans and Smith (1952)	Reproductive values (Left eigenvector)
Egg	58.91	67.88	1.00
1st instar (L1)	18.97	15.18	3.17
2^{nd} instar (L2)	8.05	6.99	5.88
3^{rd} instar (L3)	6.00	4.26	8.92
Adult	8.07	5.69	14.78

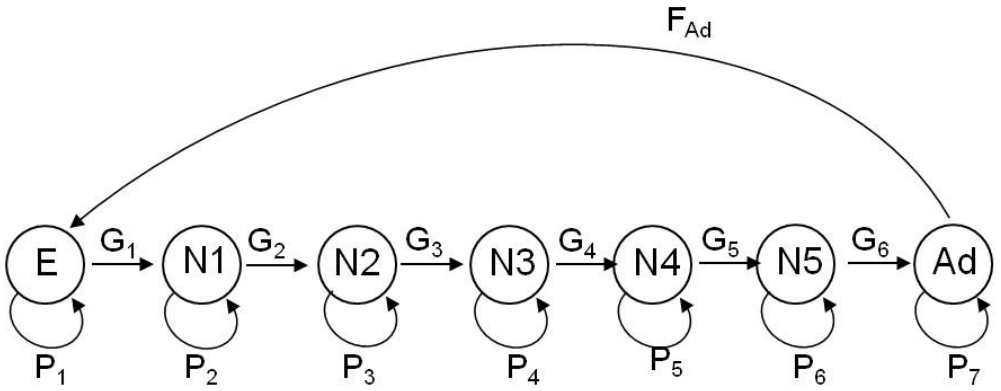

Figure 6.1. Coates life cycle graph of the seven stages of the common bed bug, *Cimex lectularius*. *E* = egg stage, *N1* to *N5* the five larval stages, and *Ad* is the adult stage; $G_i = S_iD_i$, the probability of individuals in the *i*th stage surviving (S_i) and developing (D_i) to the next stage; $P_i = S_i(1 - D_i)$, the probability of individuals in the *i*th stage surviving (S_i) and not developing $(1 - D_i)$ to the next stage; F_{Ad} is female fecundity.

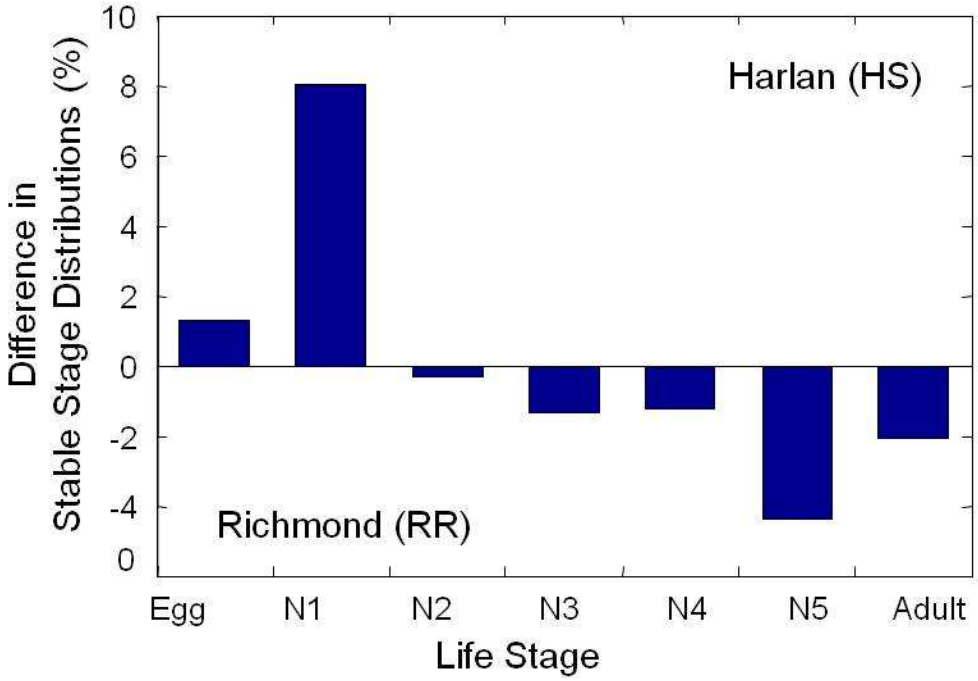

Figure 6.2. Stage-specific differences between the stable stage distributions generated from the projection matrices for the Harland and Richmond bed bug strains. The zero line represents no difference between the two strains in the percentage of the respective life stage in the population at the stable stage distribution.

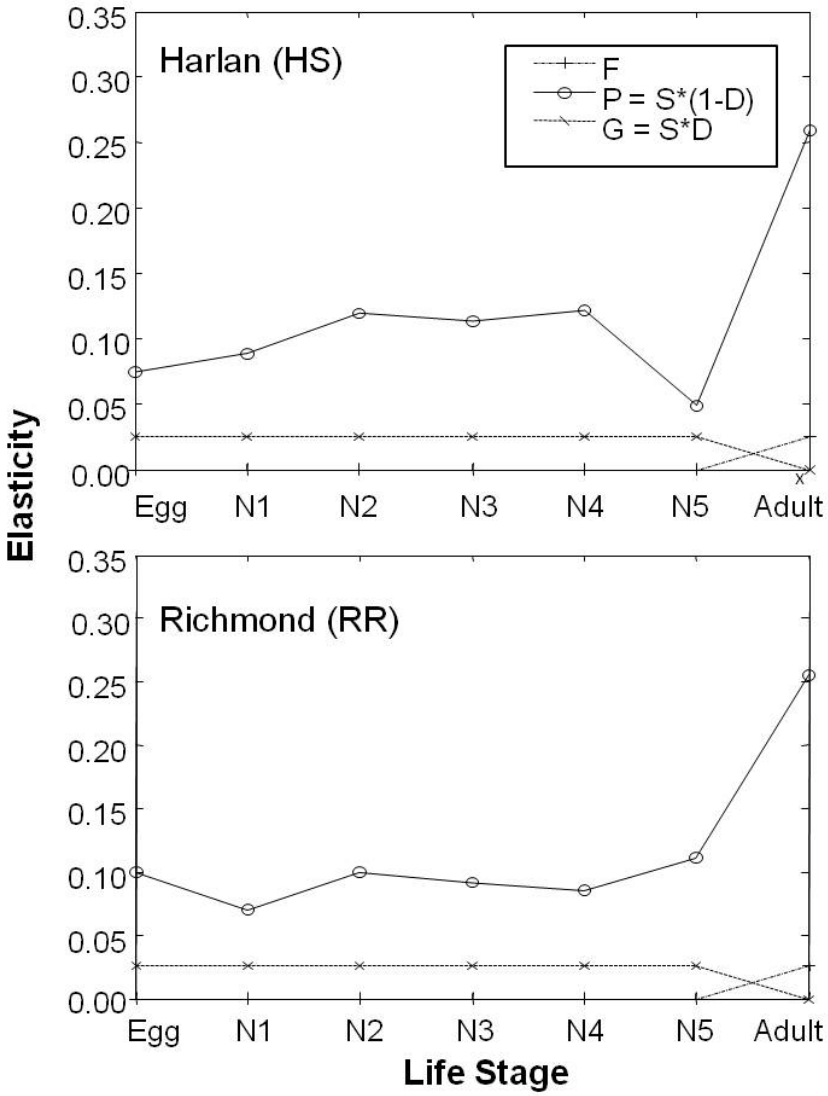

Figure 6.3. Elasticity or proportional sensitivity of λ_m to changes in fecundity (F_A), survival and not development to the next stage (P_i), and survival and growing to the next stage (G_i) for the two bed bug strains. The elasticities of the matrix elements sum to 1.0 and therefore, they can be compared directly to determine their contribution to the population growth rate.

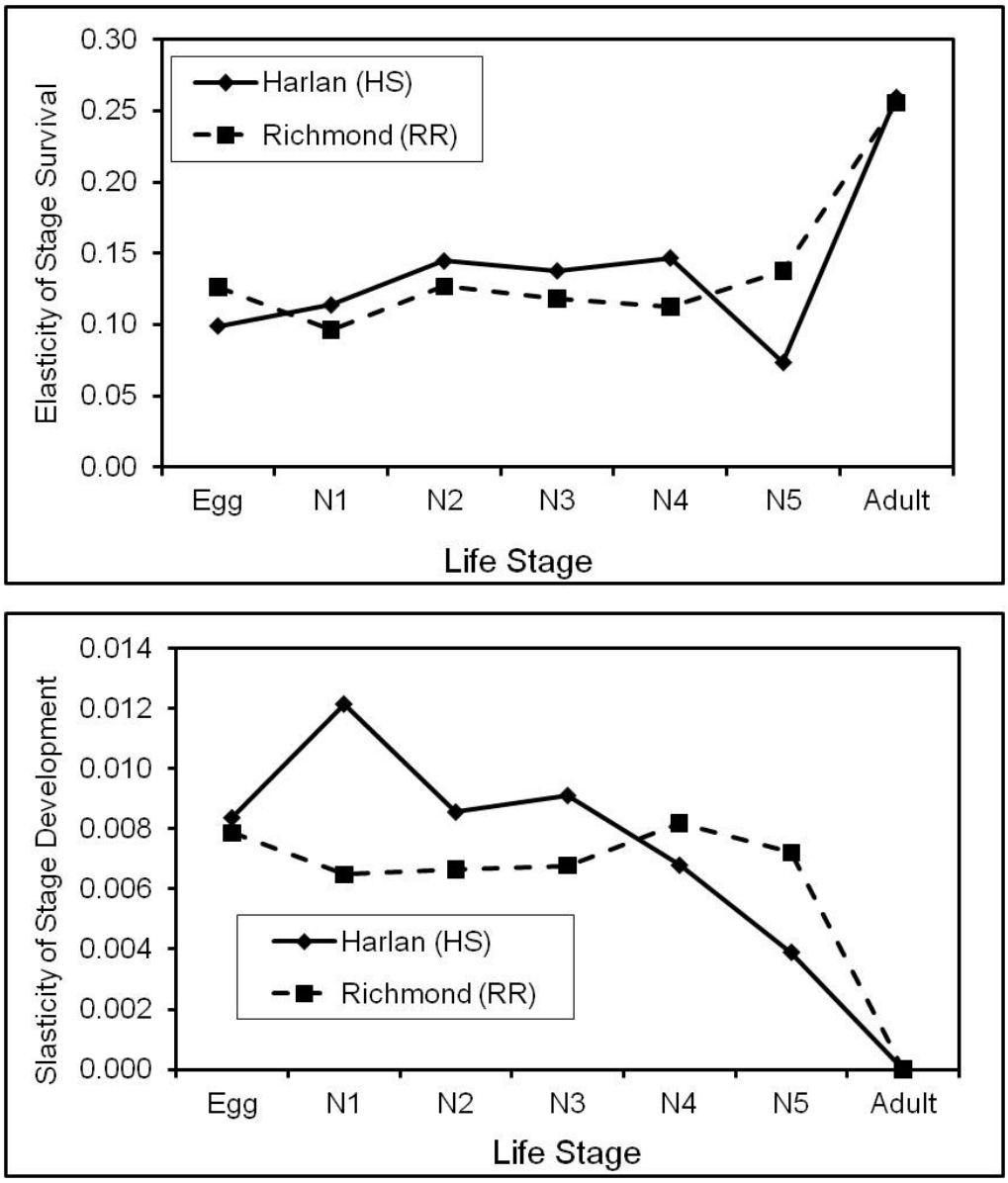

Figure 6.4. Elasticity or proportional sensitivity of λ_m to changes in stage-specific survival probability, S_i (top) and to stage-specific development, D_i (bottom) for the two bed bug strains.

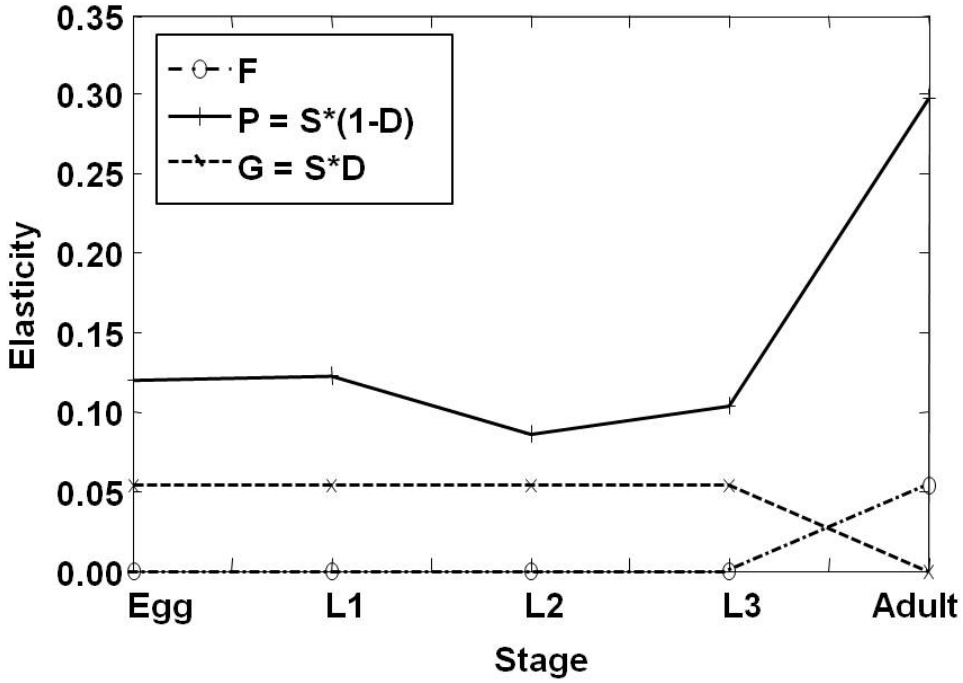

Figure 6.5. Elasticity or proportional sensitivity of λ_m to changes in fecundity (F_A), survival and not development to the next stage (P_i), and survival and growing to the next stage (G_i) for the human louse. The elasticities of the matrix elements sum to 1.0 and therefore, they can be compared directly to determine their contribution to the population growth rate.

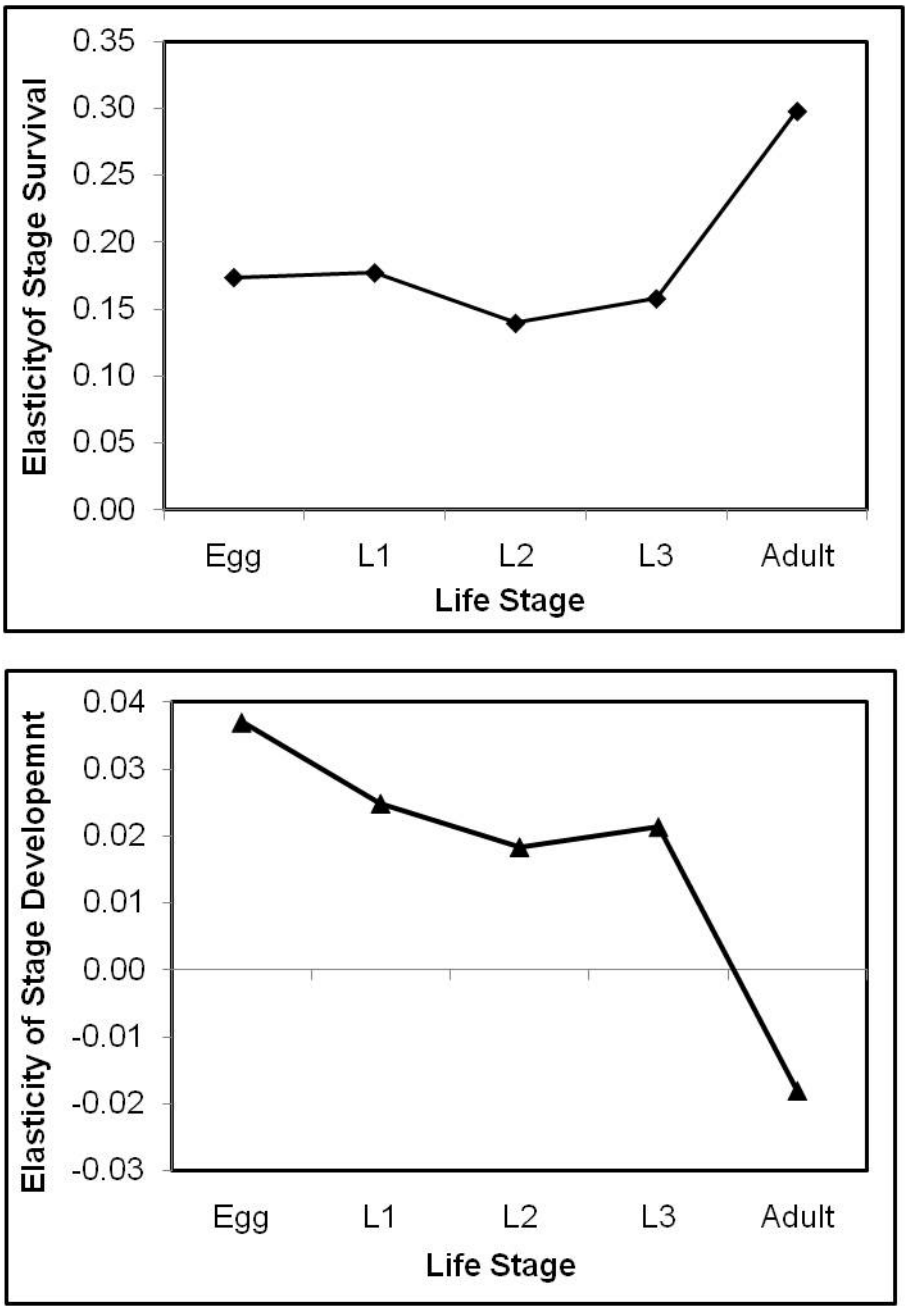

Figure 6.6. Elasticity or proportional sensitivity of λ_m to changes in stage-specific survival probability, S_i (top) and to stage-specific development, D_i (bottom) for the human louse.

Chapter 7: Summary

In order to control modern bed bug populations, a better understanding of the ecology and biology of the insect is required. The general features of the biology of *C. lectularius* was described many years ago (Usinger 1966), as were the most significant aspects of the ecology of the insect (Gunn 1933, Jonhson 1940, Davis 1964). Since bed bug populations were almost eradicated from the U.S. there is a gap in the scientific bed bug research between the 1940's and today (2010).

To date very little information is available on the ecology and life history of bed bug populations currently infesting the U.S, and on management tactics that are specific to different infestations. We know little about the fecundity, survivorship, and dynamics of modern bed bug populations. The different conditions under which modern bed bug populations have evolved could have had a significant effect on their development and fecundity. Developing life tables and mathematical models for modern bed bug populations will help quantify changes in the development, survivorship, and fecundity of populations under specific conditions. Life tables allow also for comparison of life history parameters between pesticide resistance and susceptible bed bug populations. Therefore, six bed bug strains, four resistant to pyrethroids and two susceptible, were chosen to study the development, survivorship and fecundity of the species under laboratory conditions.

When evaluating bed bug fecundity, it was found that females produced 131.9 (± 2.57 SE), 138.5 (± 2.43 SE), and 155.6 (± 2.41 SE) mean number of eggs during the 13 feeding/oviposition cycle study for RR, NR, and ER strains respectively. All the females from the RR strain died at the end of the 13 feedings. At the end of the study 15 of the 20 females from the ER strain had died. 13 out of 20 females from the NR strain were dead at the end of 13th

feeding. The overlapping confidence intervals indicate that there were no significant differences among the mean number of eggs oviposited by the three strains of bed bugs over the course of the study. However, significant differences ($F = 7.32$; df = 24, 7140; $P < 0.0001$) were observed in egg production pattern for a particular feeding among the three strains. The ER strain started producing eggs later and peaked later in comparison with the other two strains. In this study, the egg production decreased drastically after the 12^{th} and 13^{th} feeding/oviposition cycles. This decrease in egg production can be explained because as females age, they become less fertile (Johnson 1940). In addition, the majority of females died after 13 feeding/ovipositions. This mortality can be attributed to the fact that repeated copulation can be energetically costly for females (Stutt and Sive-Jothy 2001).

When evaluating bed bug survival time during starvation it was found that individuals from all the strains (HS, BS, ER, RR) survived a maximum average of 128 days. The maximum individual survivorship was recorded at 135 days from the BS strain. Survivorship in *C. lectularius* has been studied by many researchers (Bacot 1914, Kemper 1930, Johnson 1941, Omori 1941, Gunn 1949) under different environmental conditions and each study provides different results about how long a bed bug can live without a blood meal. However, it is very interesting how in this study the two susceptible strains lived significantly longer than the resistant bed bug. The significant differences in survivorship after starvation between susceptible and resistant strains can be the consequence of a fitness trade-off in resistant populations. Fitness in populations of insects resistant to insecticides is affected in terms of fecundity and survivorship in absence of the insecticide. It is thought that the alleles that confer resistance to insecticides are initially present in the insect's genotype at some sort of mutation-selection

balance, and that they are slightly deleterious when there is not exposure to insecticides (Georghiou and Taylor 1986).

The purpose of the third study was to document and quantify the biological attributes of selected bed bugs strains. These attributes included survivorship and development of the immature stages, and adult survivorship and fecundity. These biological attributes were summarized in the form of cohort life tables to reflect the stage transition dynamics of the insect under specific environmental conditions. A survivorship life table was developed for resistant field strains (RR and KR), and the laboratory susceptible strain (HS), using the procedures described in Carey (1993). In addition, a complete reproductive life table for the resistant field strain (RR) was developed using the methods described by Leslie and Park (1949). The RR resistant bed bug strain had greater survivorship (egg to adult) and a faster developmental time (egg to adult) than the susceptible strain. These results were interesting because most resistant populations have a reduction in longevity and speed of development due to the amount of energy invested in insecticide detoxification (Georghiou and Taylor 1986, Uyenoyama 1986).

The reproductive life table showed that bed bugs from our experiments had an intrinsic rate of increase of 0.0453, which means that the bed bug population is increasing at a rate of 0.043 individuals per female per day. A population increasing at the rate of 0.04 per individual per day would double in number every 16.11 days. R_o, the net reproductive rate, indicates that one live female egg would on the average be replaced by approximately 35 live daughter eggs, that is, there is a 35-fold increase of females per generation.

The purpose of the final study was to develop stage-classified projection matrix models to gain insight into the population characteristics and dynamics of two bed bug strains. The first strain is one that has been shown to be highly susceptible to pyrethroid insecticides. The other

strain is one that is highly resistant to the same insecticides. We used data from life tables to develop the models and to determine population characteristics such as the intrinsic rate of increase, the stable age distribution, reproductive values, and sensitivity of the population growth rate to changes in the survival and development of individual life stages. We also developed and analyzed a projection matrix model for another parasitic insect of human, the human louse, *Pediculus humanus* L., using life table data presented in Evans and Smith (1952). We found that the two populations of bed bugs had positive growth rates, which means that the populations are projected to grow if the conditions under which the studies were conducted were held constant. The doubling times in the population indicated that under similar environmental conditions it would take a population of the RR bed bug strain ≈7 days longer to double in size compared with the HS strain. The stable stage distributions of both strains were dominated by the egg stage, which comprised >25% of the populations. Approximately 55% of individuals in the stable stage population for the HS strain are in the nymphal stages (N1 to N5) compared with 64% for the RR strain. However, there was no significant difference between the stable stage distribution of the two strains ($\chi 2$= 9.0066, df = 6, P = 0.1732). No significant difference was found in the expected reproductive contribution of the various life stages to future population size between the two strains ($\chi 2$= 1.5458, df = 6, P = 0.9564). However, the reproductive contributions of life stages other than eggs were generally higher for the HS strain than for the RR strain. A similar trend of increasing reproductive values from egg to adult stage was obtained for the human louse.

For both bed bug strains, λ_m was least sensitive to changes in adult fecundity (F_{Ad}). The sensitivity of λ_m to changes in the probability of surviving and developing, G_i, was similar for the two strains. λ_m , however, appeared to be most sensitive to changes in the probability of survival while remaining in the stage, P_i. Overall, for both strains, changes in P_i for the adult stage are

expected to have the greatest impact on λ_m relative to changes in P_i for the other life stages. It is clear from these analyses that the key to the reduction of the populations of bed bugs lies with the reduction of survival of the adults. However, there are several reasons why management of the insect has so far not been effective. The reasons include the cryptic behavior of the insect, which reduces contact with insecticides, the lack of residual activity of the applied insecticides, and lack of economical, safe, and effective alternatives, and lack of efficient sampling methods for the insect.

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