COMPARATIVE STUDIES ON CULTURE METHOD, BIOLOGICAL PARAMETERS, AND CLIMBING ABILITIES OF TROPICAL BED BUG, *Cimex hemipterus* (F.) AND COMMON BED BUG, *Cimex lectularius* L. (HEMIPTERA: CIMICIDAE)

KIM DAE YUN

UNIVERSITI SAINS MALAYSIA

2017

COMPARATIVE STUDIES ON CULTURE METHOD, BIOLOGICAL PARAMETERS, AND CLIMBING ABILITIES OF TROPICAL BED BUG, *Cimex hemipterus* (F.) AND COMMON BED BUG, *Cimex lectularius* L. (HEMIPTERA: CIMICIDAE)

by

KIM DAE YUN

Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

December 2017

ACKNOWLEDGEMENT

First, I would like to thank my supervisor, Prof. Lee Chow Yang and his family. Especially, his unwaverly support, both intellectually and financially on my research. I have learnt and experienced a lot from my stay in Malaysia, and conducting research in the world renowned urban entomology laboratory in Universiti Sains Malaysia.

Next, my deepest gratitude to my dear wife, Helena who has helped with the funding of my tuition fee for many years during my candidature in USM. Sorry for being a "human bed bug" to you.

Thanks are also due to the former students of the Urban lab who are now operating pest management companies had helped with bed bug research in the field. Regine Lim, Lim Kay Tee, Annie Eow and Alex provided various sampling sites. Also, to the collaborators in Sydney Westmead hospital, Dr. Stephen L. Doggett, Dr. Cameron Webb and Dr. David Lilly who had provided multiple strains of common bed bugs.

When we arrived in Penang, Dr. Ching-Chen Lee picked us up from the airport and helped us find a place to live. Dr. Tee Hui Siang shared plenty of his time with us during the purchase of a second-hand car, applying for driver's license, etc. Both helped us a lot so that we could be settled down within very short time.

Lastly, to all my other seniors and juniors of the Urban Lab, the staff of the School of Biological Sciences whom I could not list them all out here, thank you very much for your help in one way or another. I am truly grateful for what you have done to make my research progressed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CCD	Charged Coupled Device
df	degrees of freedom
DRB	Defibrinated Rabbit Blood
EDTA	Ethylene-Diamine-Tetra-Acetic acid
e.g.	exempli gratia, for example
et al.	et alii, and others (people)
GL	*C. hemipterus* Greenlane strain (Resistance)
HEPA	High-efficiency particulate air
HRB	Heparinized Rabbit Blood
HSD	Honest Significant Difference
i.e.	id est, in other words
IPM	Integrated Pest Management
KL	*C. hemipterus* Kuala Lumpur strain (Resistance)
MH	*C. lectularius* Monheim strain (Susceptible)
MM	*C. hemipterus* Madam Mo strain (Less resistance)
MVFF	Mean Vertical Friction Forces
PNG	*C. hemipterus* Papua New Guinea strain (Curated)
QSL	*C. hemipterus* Queensland strain (Resistance)
RH	Relative Humidity
SE(M)	Standard Error (of the mean)
SEM	Scanning Electron Microscope
SYD	*C. lectularius* Sydney strain (Resistance)
TT	*C. hemipterus* Tanjung Tokong strain (Resistance)

KAJIAN PERBANDINGAN TERHADAP KAEDAH PENGKULTURAN, PARAMETER BIOLOGI, DAN KEUPAYAAN MEMANJAT PADA PEPIJAT TROPIKA *Cimex hemipterus* (F.) DAN PEPIJAT UMUM *Cimex lectularius* L. (Hemiptera: Cimicidae)

ABSTRAK

Tesis ini berfokus pada kesan sistem pemberi makanan buatan dan semulajadi ke atas parameter biologi pepijat umum (*Cimex lectularius* Linnaeus) dan pepijat tropikal (*Cimex hemipterus* [Fabricius]), serta keupayaan kedua-dua spesies tersebut dalam memanjat perangkap jebak. Keberkesanan sistem pemberi makanan tiruan (Hemotek® membrane feeding system) dengan menggunakan dua jenis darah arnab (defibrin [Defibrinated Rabbit Blood, DRB] dan heparinized [Heparinized Rabbit Blood, HRB]) terhadap *C. lectularius* (strain Monheim [MH] dan Sydney [SYD]) dan *C. hemipterus* (strain Madam Mo [MM], Tanjung Tokong [TT], Queensland [QSL] dan Kuala Lumpur [KL]) telah dibandingkan dengan sistem pemberi makanan semula jadi, iaitu menggunakan manusia sebagai perumah. Kesan-kesan pada parameter biologi (kadar pemakanan, amaun darah yang dihisap, kadar kemunculan serangga dewasa, panjang badan, kelebaran badan, jarak antara mata, bilangan telur yang dihasilkan serta kadar penetasan telur) terhadap dua spesies dibandingkan. Tiada perbezaan yang nyata antara DRB dan HRB ($P > 0.05$) pada parameter biologi untuk semua strain pepijat serta spesies yang dikaji. Apabila dikaji pada perumah manusia, keputusan yang sama telah didapati kecuali pada strain rentan Monheim yang mengeluarkan lebih banyak telur apabila diberikan darah manusia sebagai makanan berbanding dengan DRB dan HRB ($P < 0.05$). Seterusnya, keupayaan memanjat kedua spesies pepijat, iaitu *C. lectularius* L. dan *C. hemipterus* (F.) telah dinilai dengan

menyiasat kadar perlepasan daripada perangkap jebak yang mempunyai permukaan yang licin dengan perangkap komersial Berbeza dengan *C. lectularius*, *C. hemipterus* dewasa boleh melepaskan diri daripada semua perangkap. Manakala, bilangan nimfa yang boleh melepaskan diri daripada perangkap adalah rendah atau tiada sekali. Pemeriksaan daya geseran menegak yang dimiliki oleh serangga dewasa kedua-dua spesies telah menunjukkan bahawa serangga dewasa *C. hemipterus* mempunyai daya geseran menegak yang lebih tinggi berbanding dengan *C. lectularius*. Pemerhatian imej mikroskopi elektron pengimbas pada pad tibia pepijat dewasa *C. hemipterus* telah menunjukkan terdapat lebih banyak rambut tenent pada pad tibia dewasa *C. hemipterus* berbanding dengan *C. lectularius*. Tiada pad tibia ditemui pada serangga instar ke-empat dan instar ke-lima pada kedua-dua spesies. Berdekatan dengan tapak rambut tenent yang berongga terdapat epitelium kelenjar yang berkembang dengan lebih baik pada serangga dewasa *C. hemipterus* berbanding dengan *C. lectularius*.

COMPARATIVE STUDIES ON CULTURE METHOD, BIOLOGICAL PARAMETERS, AND CLIMBING ABILITIES OF TROPICAL BED BUG, *Cimex hemipterus* (F.) AND COMMON BED BUG, *Cimex lectularius* L. (HEMIPTERA: CIMICIDAE)

ABSTRACT

This thesis focuses on the effects of artificial and natural feeding system on the biological parameters of both common (*Cimex lectularius* Linnaeus) and tropical bed bugs (*Cimex hemipterus* [Fabricius]), and the climbing ability of both species on pitfall style monitoring traps. The feeding effectiveness of an artificial feeding system (Hemotek® membrane feeding system) for *Cimex lectularius* (Monheim [MH] and Sydney [SYD] strains) and *C. hemipterus* (Madam Mo [MM], Tanjung Tokong [TT], Queensland [QSL] and Kuala Lumpur [KL] strains) using two types of rabbit blood (defibrinated [Defibrinated Rabbit Blood, DRB] and heparinized [Heparinized Rabbit Blood, HRB]) were compared with natural feeding on a human host. The effects on biological parameters (feeding rate, amount of blood intake, adult emergence rate, body length, body width, and distance between the eyes, number of eggs produced and egg hatchability rate) of both species were compared. Results found that there was no significant difference ($P > 0.05$) between DRB and HRB, on the biological parameters of all the bed bug strains and species tested. When tested on a human host, similar results were obtained, with exception to Monheim susceptible strain producing significantly ($P < 0.05$) more eggs on human blood compared to those of DRB and HRB. Next, the climbing abilities of two bed bug species, *Cimex lectularius* L. and *Cimex hemipterus* (F.) were determined by evaluating their escape rates from smooth surface pitfall traps using four commercial bed bug monitors (Verifi™ Bed Bug

Detector, ClimbUp® Insect Interceptor, BlackOut™ Bed Bug Detector, and SenSci Volcano™ Bed Bug Detector). Unlike *C. lectularius*, adult *C. hemipterus* were able to escape from all traps. On the other hand, no or low number nymphs of both species escaped, depending on the evaluated traps. Examination on the vertical friction force of adults of both species revealed a higher vertical friction force in *C. hemipterus* than in that of *C. lectularius*. Scanning Electron Micrograph (SEM) observation on the tibial pad of adult bed bugs of *C. hemipterus* showed the presence of a greater number of tenent hairs on the tibial pad than on those of adult *C. lectularius*. No tibial pad was found in the fourth and fifth instars of both species. Near the base of the hollow tenent hairs is a glandular epithelium that is better developed in adult *C. hemipterus* than in those of adult *C. lectularius*.

CHAPTER ONE

GENERAL INTRODUCTION

The resurgence of the modern bed bug (*Cimex* spp.) which has returned back in late 1990s with high pesticide resistant level (e.g. Pyrethroids) from eradication by DDT in 1940~1950's given rise to economic and medical issues worldwide. Infestations have been reported in hotels, shops (Volentine 2016), libraries (Cleveland 2017), cinemas (Anderson 2017), and even schools (Lewis 2017). Although bed bugs are known as potential vectors of Hepatitis B (Blow et al. 2001), Chagas (Salazar et al. 2015), and Ebola (Quarles 2015), no cases of bed bugs transmitting these diseases in the field have been reported to date. Bed bug bites can cause various cutaneous symptoms (Doggett 2012), systemic reactions (Minocha et al. 2017), or anemia (Pritchard and Hwang 2009). However, some people, especially the elderly, show no symptoms (Potter et al. 2010) or have a delayed reaction (Sansom et al. 1992).

The common bed bug *Cimex lectularius* L. is widely distributed in tropical and non-tropical countries, whereas the tropical bed bug *Cimex hemipterus* (F.) was confined to tropical countries (Service 2000) for a long time. However, increasing international trade and travel along with rapid transportation (Doggett et al. 2003) and comfortable living conditions (Omori 1941, Usinger 1966, Doggett 2012) have allowed *C. hemipterus* to disperse into some non-tropical countries (Doggett 2012, Campbell et al. 2016, Komatsu et al. 2016). For example, several cases of infestation by the tropical bed bug have been reported in the Asia-Pacific region (How and Lee 2010a, Tawatsin et al. 2011, Campbell et al. 2016), and Europe (Gapon and ΓΑΠΟΗ 2016). To date, however, most published research has focused on the common bed bug

(How and Lee 2010b), which is the main species present in developed countries. This has led to monitoring traps targeted only for *C. lectularius* (Thompson 2017). Lack of attention to tropical bed bugs may ultimately lead to financial losses due to damage caused by infestation in areas without effective means to control these pests.

The bed bug pest problem is characterized by high levels of infestation, and distribution can be very broad due to the transfer of bed bugs due to social activities or traveling (Baumann 2002, Massis and Gondek 2011). Additionally, high control costs (Potter et al. 2013) can lead to people not to report infestations, thereby allowing them to get worse (Pinto et al. 2007), or to attempt self-treatment (Seidel and Reinhardt 2013).

Bed bug infestation and treatment can result in the vicious cycle, and public education (Bennett et al. 2016) and early detection (Doggett 2013a) are crucial to preventing bed bugs infestation (Bed Bug Foundation 2016). Visual inspection is commonly used when an infestation is reported (Potter et al. 2015), but this approach has limitations. For example, when the infestation level is low, it can hard to locate concentrated harborages. Sniffer dogs can be useful for detecting the presence of bed bugs, but training and maintenance (Doggett 2011) and false detection (Cooper et al. 2014) is significant issue with this approach. Monitoring traps is a good option for detecting low-level bed bug infestations (Cooper et al. 2015). However, most of the commercial pitfall traps have been designed and tested only for common bed bugs. The detailed investigation against tropical bed bugs is urgently wanted.

Natural feeding on various animal or human hosts was used at most bed bug research laboratories in the world. However, this *in vivo* feeding system has limitations. The natural feeding method can cause discomfort to the human host; besides

increasingly stringient animal ethic rules could slow down the approval process (Animal Welfare Board 2015). It can even cause the death of animal hosts during long periods of bed bug research.

Today many types of *in vitro* feeding devices are available, and most use a similar feeding mechanism - heating up the blood to attract bed bugs to the feeder, which is covered by Parafilm M. However, to what impact these *in vitro* system has on the biological parameters of the bed bugs remain little known, as the choice of blood source varies between different laboratories.

The objectives of this study were:

1) to develop an *in vitro* feeding system suitable for long-term rearing of laboratory populations of both common and tropical bed bugs.

2) to evaluate the effects of artificial feeding on the biological parameters of both species of bed bugs; and

3) to compare the performance of commercially available pitfall traps against both species of bed bugs, and to determine the reasons for the lack of performance if there is any.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of bed bug

About 14,000 species from various orders such as Lepidoptera, Siphonaptera, Diptera, Hemiptera, and Phthiraptera are classified as blood sucking insects (Adams 1999), and around 300-400 of them commonly feed on humans (Lehane 2005). Order Hemiptera contains about 100 species of mammal and bird ectoparasites (Askew 1971) in families Cimicidae, Plyctenidae, and Reduviidae (specifically subfamily Triatominae). Polyctenids are small eyeless and wingless insects that feed on bats, thus they are not economically or medically important for humans (Lehane 2005). However, the Triatominae (kissing bugs) and Cimicidae (bed bugs) contain human ectoparasite species. All members of the Cimicidae feed on blood (Lehane 2005), but they are ectoparasites and do not live in hosts or under the skin, unlike endoparasites (Askew 1971, Russell et al. 2013). Most Cimicids are bat or bird parasites (Usinger 1966), but three species feed on human blood, namely *Cimex lectularius* L., *Cimex hemipterus* (F.), and *Leptocimex boueti* (Brumpt) (Russell et al. 2013). *C. lectularius*, or common bed bugs, live in temperate and subtropical regions and feed on humans, bats, chickens, and other domestic animals. *C. hemipterus*, or tropical bed bugs, inhabit tropical and subtropical regions and feed on humans and chickens. *L. boueti*, or African bed bugs, live in West African regions and feed on bats and humans.

Bed bugs are obligate hematophages, thus nymphs and adults consume blood as their only food (Johnson 1952, Lehane 2005). All five nymphal instar stages require blood meals in order to molt to the next stage (Lehane 2005, Russell et al. 2013). If

the amount of blood ingested is not sufficient, the nymphal development periods can be delayed (Kemper 1936, cited by Usinger 1966, Johnson 1952). Females must feed prior to mating and produce eggs (Johnson 1952), and males require feeding before producing sperm and seminal fluid (Usinger 1966, cited by Reinhardt and Siva-Jothy 2007). As the bed bugs fully fed, immediately back to harborage till digest which takes a few days - depends on temperature, but they can survive without food for more than a year (Pinto et al. 2007). Mated females lay fertilized eggs on rough surfaces and attach them by secreting a transparent sticky cement. Eggs hatch after about a week, but the incubation period depends on the indoor temperature (Omori 1941), which must be between 13 and 37^{o}C (Russell et al. 2013) for hatching to occur. Omori (1941) reported that bed bugs showed better biological parameters (e.g., fecundity, hatchability, and emerging rates) when the indoor temperature was constant rather than fluctuating (i.e., outdoors). This phenomenon explains why bed bugs stayed inside caves in the Middle East, which had constant temperatures (about 22-24^{o}C, Anciaux de Faveaux 1959, cited by Usinger 1966) throughout the year and provided regular feeding opportunities on bats or early humans (Usinger and Povolny 1966). Agricultural evolution caused humans to leave caves for tents and houses. Modern habitation sites have central heating systems (Bean and ArchD 2010), which act as artificial caves for bed bugs. Over time, activities such as national or international trading helped spread the distribution of bed bugs throughout the world (Johnson 1941). This phenomenon raised bed bugs to ‘pest' status.

C. lectularius and *C. hemipterus* are the two main modern bed bugs that feed on humans. They have a similar range of preferred temperatures (28-30℃) (Omori 1941). If they co-exist in the same location, cross-mating could take place (Omori 1941,

Newberry et al. 1987), but the hybrids produced by both species will be sterile (Newberry 1988). Omori (1941) suggested that the two bed bugs species naturally have different geographical distributions because of interspecific competition, but recent globalization is leading to mixing of the species (Tawatsin et al. 2013). As both bed bug species are thigmotactic, they prefer their body in contact with rough surfaces such as cracks and crevice of walls, paints, mattress seams, bed frames, and screw holes (Figure 2.1) (Kemper 1936, cited by Usinger 1966, Usinger 1966, Russell et al. 2013). This is one of the reasons why detecting bed bugs during the daytime, especially the small nymphs, is difficult. When digesting recently consumed blood meals, bed bugs rest in the harborage, which contains feces, egg shells, and empty skins from various stages of nymphs (Usinger 1966, Choe et al. 2016).

Unlike passive dispersal, in which bed bugs hitchhike on a host's belonging (Kemper 1936, cited by Usinger 1966, Paul and Bates 2000, Doggett et al. 2003, Kells 2006), bed bugs will leave the harborage via active dispersal for several reasons, such as searching for a host for feeding (How and Lee, 2010c), being disturbed by a pesticide (Romero et al. 2009), exiting a harborage that is too crowded (Wang et al. 2010), or mating (Pfiester et al. 2009). Adult bed bugs exhibit a unique mating behavior called ‘traumatic insemination' (Siva-Jothy 2006), so named because the female is damaged during the mating process, which results in a shortened lifespan. To avoid multiple mating, female bed bugs leave the male dominated harborage site to find a new harborage in which to oviposit eggs after feeding and mating (Pfiester et al. 2009, How and Lee 2010c).

Peak feeding time for bed bugs is before sunrise (Mellanby 1939, Usinger 1966), and they are attracted to a host mainly by its warmth (body heat), carbon dioxide

emission (Hase 1917, cited by Usinger 1966, Russell et al. 2013). Host-seeking mechanisms have been studied since the early 1900s and included tests using various hosts, including cold blood reptiles such as geckos, lizards, and snakes (Chatton and Blanc 1918, Rivnay 1930). In general, the host should be at least 2℃ warmer than the indoor temperature in order to attract bed bugs. Nymphs take about 24 hours before they are ready to feed post hatching or molting (Russell et al. 2013).

According to Askew (1971), the threshold temperature for adult activity and egg-hatching of the common bed bugs is 13-15℃, but it is higher for tropical bed bugs. Both species can tolerate a wide humidity range (Askew 1971). These temperature requirements explain why tropical bed bugs are dominant in tropical regions (Horváth 1914, cited by Usinger 1966) and common bed bugs are widespread in the Northern Hemisphere (Russell et al. 2013). When living in the temperate, common bed bugs may produce one and a half generations per year (Askew 1971), while tropical bed bugs produce more (> 10 generations) in the tropics. This phenomenon can explain why the common bed bugs developed more in most part of the biological parameters such as fecundity, longevity including meal size influenced by body size than those of tropical bed bugs. However, the common bed bugs originally moved to northern territories from warmer parts of the Palearctic as indoor heating systems improved (Askew 1971). This phenomenon caused rapid and serious infestation problems in large towns and seaports. For example, in 1939 more than 4 million people in London suffered from bed bugs infestations, and it led to the development of the pest management industry (e.g. ‘Tiffin and Son', which is still in operation today for > 100 years).

Use of powerful pesticides such as DDT as well as improved regulations related to sanitation had mostly eradicated bed bugs by the 1970s (Askew 1971). However, improved transportation system and world economic growth increased international tourism and trade, especially in tropical countries such in Southeast Asia. This development led to widespread dispersal of both common bed bugs and the tropical species. The tropical bed bug (i.e., a new invasive species) is quickly spreading to many parts of worlds, including Australia (Doggett et al. 2003), Korea, China, Japan, Russia, and Europe. This phenomenon requires expanding the scope of scientific studies of the common bed bug to include tropical bed bugs.

Figure 2.1 Harborage of *Cimex hemipterus* located in screw hole of bed frame.

2.2 Morphology of bed bug

Common and tropical bed bugs have several distinguishing features. Female *C. lectularius* are 4.5-5.0 mm in length, reddish brown (chestnut) in color, and covered with fine golden hairs. Males usually are smaller than females. Common bed bugs have two well-developed eyes that are prominent laterally and four segmented antennae that detect heat and pheromones (the first two near the head are thicker than the third and fourth, which are slender and covered with long hairs and sensors). A wide (2.5 times the length compared to the middle height) and curved prothorax covers

half of the head and has a rounded edge that extends up to eye level. In *C. lectularius* this edge is flat and forms wings around the whole prothorax, which is not the case for *C. hemipterus* (Figure 2.2). In addition, each species has different egg shell surface patterns (Figure 2.4). Thus, these characteristics are distinctive morphological differences between the two species (Usinger 1966).

The first pair of legs is on the ventral surface of the prothorax, whereas the middle and hind legs are on the mesothorax and metathorax, respectively. Dorsally, the mesothorax has a triangle shaped scutellum that points downward, and two hemelytral pads are located laterally, but absence of wings. The abdomen has 11 segments, but only 2 to 9 are observed dorsally, with a rounded shape for the whole body of male *C. lectularius* and a sharp shaped body for male *C. hemipterus* (Figure 2.3). If a bed bug is fully engorged, the 'hunger fold', which consists of intersegmental membranes, will be exposed, and body volume and size will be increased. Seven pairs of spiracles are located ventrally on segments 2 to 8. Females have an ectospermalege (or paragenital sinus or notch) located ventrally and to the right on segment 5, whereas males have a paramere on the left side at the end of the abdomen (Kettle 1984). Common bed bugs are distributed throughout Europe and North America, and also are found in Suez, Egypt, the Sudan, the North-West Frontier Province of India, China, South Africa and Australia (Patton 1908). Darker and smaller tropical species (so-called Indian bed bugs or tropical bed bugs) are distributed throughout India, Burma, Assam, and Malay and also occur in Aden, Sierra Leone, the Islands of Mauritius, Reunion, St. Vincent, and Puerto Rico (Patton 1908).

According to Wigglesworth (1939) and Chapman (1998), the crawling insects are using two different methods for walking on rough or smooth surfaces. Tarsal claws

commonly used for gripping rough surfaces. However, on the smooth surfaces, insects are using adhesive organs because too smooth to holding surface using claws. The adhesive organs shaped spongy or hairy which has expanded and moistened pad on the tip so called tenent hair or *fossula spongiosa* which have various shapes and mechanisms depends on insect order, family and purpose (Wigglesworth 1938). Basically, the tenent hairs exist for climbing up on the smooth surfaces such as leaf, but for predators (e.g. Reduviidae), they are using the tibial pads as a grasping organ to catch preys with smooth body cuticles (Haridass and Ananthakrishnan 1980). There are various assumptions about the mechanism of adhesive function of insects. Recent studies explain it as cohesive forces and molecular adhesion by pad secretion, but more detailed studies still wanted (Dixon et al. 1990, Gorb 2000, Gorb and Beutel 2001, Gorb 1998a, 1998b, Hosoda and Gorb 2011, Walker et al. 1985).

Bed bug's *fossula spongiosa* is also reported by Usinger (1966) and Walpole (1987) for both common and tropical bed bugs. However, they only described it morphologically for taxonomic purposes rather than a mention about its function. Rothschild and Hinton (1968) commented that the bed bug's tenent hairs are similar with male fleas' antennae which have adhesive function. In addition, Weirauch (2007) and Green et al. (1998) pointed the bed bug's *fossula spongiosa* likely exist for the mating purpose, but nothing is investigated for its adhesive function and ability until now.

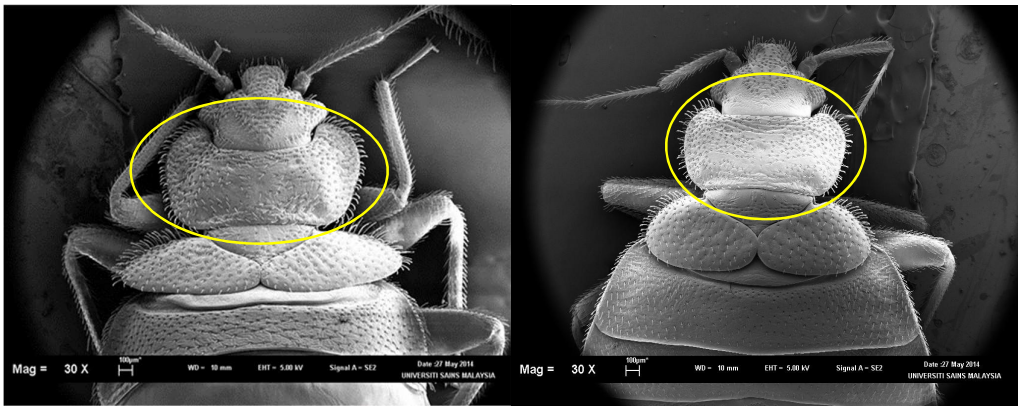

Figure 2.2 SEM micrographs (30× magnification) showing the different shape of pro-prothorax (or pronotum) of common bed bug (Left) and tropical bed bug (Right).

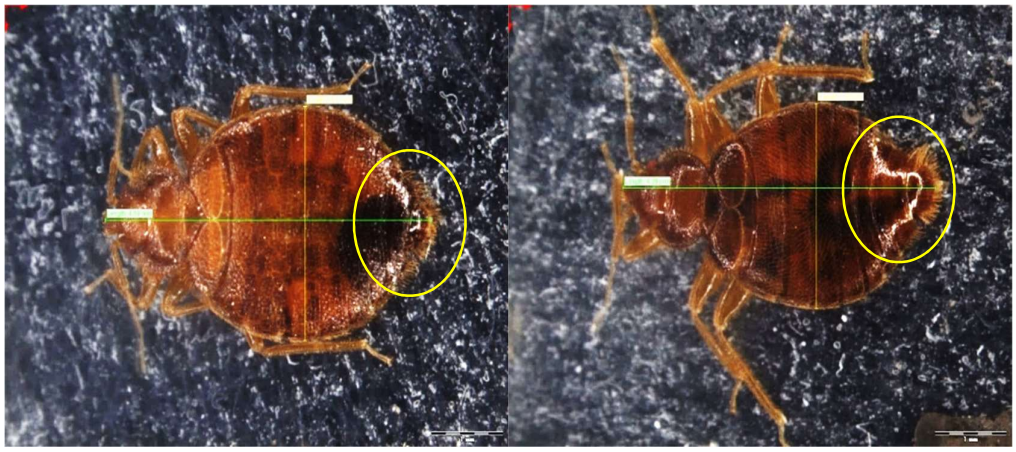

Figure 2.3 The male common bed bug's (Left) pre-genital organ is more rounded than that of tropical bed bug (Right).

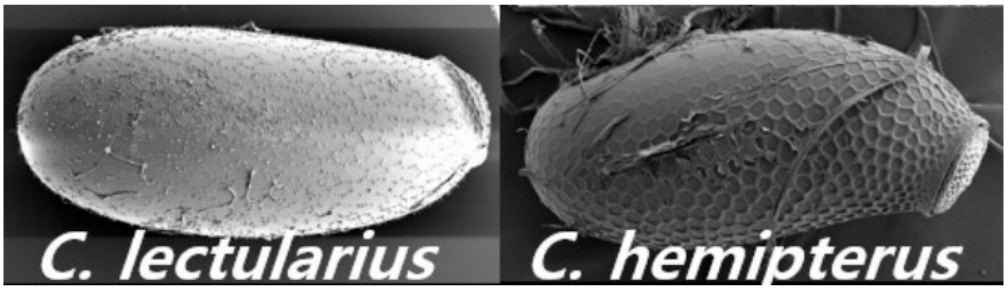

Figure 2.4 Eggs shells of the common bed bugs (Left) and the tropical bed bugs (Right).

2.3 Bed bug culture for mass production

Insect rearing (culture) is essential for providing test organisms for scientific study. Numerous studies have been conducted on a variety of pests, including household and economically important pests such as cockroaches (Clayton 1959) and termites (Lee and Lee 2015) and also blood feeding insects such as head lice (Yoon et al. 2006), mosquitoes (Deng et al. 2012), and bed bugs (Montes et al. 2002). Most studies focus on biology, foraging, and feeding behaviors. Studying biological processes such as development, reproduction, and life cycle are critical to understanding insects (Omori 1941). Knowing the target insect's biological parameters and how they relate to the environment allows researchers to utilize the best temperature and humidity to increase the insect number quickly during the culturing process.

Haematophagous insects search for hosts by detecting carbon dioxide using antennae (Gillies 1980) and also by detecting heat (Rivnay 1930, Usinger 1966) near their hosts. Using their needle-like mouth parts, blood feeding insects penetrate the skin surface to find a blood vessel (Jung et al. 2015) and feed until they are fully engorged. Bed bugs ingest a large amount of blood, which then is digested by symbionts (Sakamoto and Rasgon 2006, Rio et al. 2016) in the insect gut to obtain the nutrients (e.g. vitamin B) needed to grow up (molt out) or produce eggs (Chang 1974). All nymph stages and adults need to feed on blood to molt out, mate, and produce eggs.

Large numbers of insects are needed for research, and weekly blood feedings are crucial for maintaining or increasing the bed bug colony. For example, biological studies of bed bugs require observation of the life cycle from 1st instar nymphs to adults, and regular blood meals are required to allow them to mature. Pesticide resistance

bioassays require thousands of adults to test the effects of various active ingredients. The process of rearing however faces a number of obstacles, such as cost, effectiveness (Chin-Heady et al. 2013, Rahim et al. 2015), and the well-being of the test insects (Bryant and Jones 2012). The cost of providing regular (weekly) blood meals is high. If the blood is not suitable, it can negatively impact bed bug fecundity, nymphal development, body size, and overall health, which in turn can affect test results (Barbarin et al. 2013, Feldlaufer et al. 2014, Janisch 1933). Finally, safely of the blood meal is crucial. As Bryant and Jones (2012) noted, if the purchased blood is contaminated by pesticides or antibiotics, it can kill whole bed bug colonies with only a single feeding.

Bed bugs have been cultured for research purposes since the early 1900s. In the early years, cultured bed bugs generally were fed using human or animal hosts such as rabbits, rats, and mice when using direct (or natural) feeding methods (*in vivo*) (Janisch 1933, Wattal and Kalra 1961, United States Department of Agriculture 1963). However, bed bug bites can cause serious allergic reactions depending on the person (Minocha et al. 2017) or animal, and small-sized animals such as mice can be killed by hundreds of bed bug bites. Even though the *in vivo* feeding method has limitations and is not recommended by animal ethics committees (Animal Welfare Board 2015), over 50% of researchers still use this feeding method according to a recent 10-year survey (Aak and Rukke 2014).

To overcome the limitations of the in vivo feeding system, studies of *in vitro* (or artificial) feeding methods were conducted. However, they failed to attract bed bugs (Andre 1912, Cornwall and La Frenais 1916, both cited by Rivnay, 1930) or mouse skin (De Meillon and Golberg 1947) successfully attracted bed bugs and allowed both

nymphs and adults to suck the substrate in the feeder. Currently, artificial feeding still faces two main problems: high feeding mortality (Rahim et al. 2015) and poor quality of artificially raised insects (e.g., small body size, low fecundity, and slow development) (Aak and Rukke 2014).

Post-feeding mortality was confirmed by De meillon and Golberg (1947), who reported that aged blood showed hemolysis, which caused high mortality in bed bugs after feeding. Takano-Lee et al. (2003a) and Yoon et al. (2006) also noted that blood containing fungal or bacterial contamination resulted in high bed bug mortality. Rahim et al. (2015) recently reported that bed bug feeding on expired human blood caused high mortality (> 90%) and low emergence rate (< 1%). In other studies, Aak and Rukke (2014) and Polanco et al. (2011) described the poor quality of artificially raised insects. They found that > 70% of *C. lectularius* nymphs emerged after artificial feeding with 1% heparinized human blood. However, adults had smaller body size than naturally fed adults. In addition, artificially fed females had lower fecundity. De meillon and Golberg (1947) reported that poor quality blood meals led to lower fecundity, smaller body size, and slower development. This phenomenon indicates that blood quality is directly related to the insects' biology and lifespan. Therefore, safe and cost effective sustainable artificial feeding system for both of common and tropical bed bugs are urgently needed for long-term bed bug research in a laboratory.

2.4 Bed bug monitoring and detection

A variety of methods to trap or eliminate bed bugs have been developed. The most popular physical barrier to bed bugs in the eighteenth century was dishes filled with water or kerosene that were placed under the bed to entrap the bugs (Wright 1962 cited by Potter 2011). Other trapping methods included wood plates containing many

holes or folded fabrics put under the bed at night. The next morning, entrapped bugs will be eliminated by clean up (Busvine 1976 cited by Potter 2011). In Southeast Europe, natural traps such as the common bean leaf have been used (Bogdandy 1927, Richardson 1943). After placing leaves under the bed, bed bugs will be caught on the leaf surface by hook-like hairs (trichomes), where they will die due to starvation or desiccation. Development of this kind of technology from natural materials continues today (Robinson 2005, Szynder et al. 2013).

The pattern of modern bed bug resurgence that occurred in the 1990s looks similar to the one that took place a century ago. For instance, in the United States, popular cities such as New York, San Francisco, and Miami, which are centered around airports and hotels used by international travelers and business persons experienced a resurgence in infestations (Potter 2010). The same phenomenon occurred in China, with floating population cities such as Shenzhen near Hong Kong and the Pearl River Delta industrial region having bed bug problems while other places did not. Bed bugs then were distributed to other parts of the nation along with people's movement and with products manufactured in infested locales (Wang and Wen 2011, Wang et al. 2013). A recent study conducted in Malaysia reported that over 52% of bed bug infestation locations were foreign worker dormitories, and another 48% were airports and hotels (Zulaikha et al. 2016). Doggett and Russell (2007) reported similar results in Australia. Thus, today's dispersion of bed bugs along with travelers and immigrants is the pattern seen a century ago in Europe.

Modern bed bugs have developed resistance to most approved household insecticides (How and Lee 2011), which also contributed to the rapid distribution and resurgence of bed bugs around the world. Our lack of concern and knowledge about

bed bugs for the last 50 years, when bed bugs were mostly absent, exacerbates the bed bug problem today (Wang et al. 2010, Davies et al. 2012).

Bed bug management often fails due to ignorance of bed bug infestation at the early stage (Wang et al. 2010). In agreement with experts from 100 years ago, modern pest management professionals emphasize the importance of early detection and prevention of bed bugs. Potter (2011) mentioned that we should learn from and apply bed bug management skills and methods from historical accounts. However, most people do not understand the importance of proactive bed bug monitoring activities in the early stage of resurgence because they have had no experience with infestations for the last several decades (Potter et al. 2008). In one study, Potter et al. (2015) found that by educating people and learning from experience, over 73% of people realized and agreed with the importance of early detection.

It is difficult to prevent bed bug infestations from outside sources, but it is possible to detect an infestation early. As early detection makes management more successful, preventive inspection is important to stop population increases (Wang et al. 2009, Potter et al. 2015). In the field, all bed bug professionals conduct visual inspections, but a low infestation level increases the chance of missing harborages. Furthermore, modern houses are full of furniture, so it is difficult to check every single crack and crevice. To overcome these problems, sniffer dogs or various types of traps are used (Bed Bug Foundation 2016, Doggett 2013a).

A common mistake in visual inspection is missing bed bug harborages due to lack of time for detailed detection. During the inspection, people should be careful not to make the bed bugs disperse. For example, the home owner should never move housewares before the professional arrives. The professional should carry on the

minimum number of devices for inspecting and never sit on infested beds or sofas. They should check not only the beds, but also sub-rooms, bedside units, and up and down stairs. In the case of hotels, laundry, lounge, and cleaner's rooms should also be inspected, as they can be main points of dispersion (Doggett 2013a, Bed Bug Foundation 2016).

Sniffer dogs are very helpful for detecting low levels of infestation. However, because dogs cannot reach higher places in the room, a visual inspection should be performed at the same time. Sniffer dogs should be trained every day using live bed bugs, which means that trainers have to rear bed bugs for training purpose. However, daily training is costly, and false detection is a possibility (Doggett 2013a, Bed Bug Foundation 2016, Cooper et al. 2014). Therefore, monitoring traps are the most commonly applied method in the field for early detection and evaluation at post treatment (Potter et al. 2015, Cooper et al. 2016). In 2008, over 50% of professionals used traps (i.e., installing double sided tape on the bed legs) as a physical barrier. However, bed bugs avoid sticky surfaces, so this approach to monitoring proved to be unsuitable (Potter et al. 2008, Potter 2011, Doggett 2012).

Attractants can be used to increase trapping efficacy. For example, aggregation pheromone can be used to cause bed bugs to search for a harborage. In addition, host cues, such as carbon dioxide or heat, can be used to attract foraging bed bugs. Attractants can be applied to active traps such as pitfall or sticky traps. However, passive traps, which contain no attractants, are most commonly installed in the field (Potter et al. 2015), even though active traps perform better (Anderson et al. 2009, Singh et al. 2012). This probably is due to price, running costs, and efficacy data in the field (Bed Bug Foundation 2016). Cooper et al. (2015) evaluated passive pitfall

traps at a multi-unit apartment and confirmed that they were very useful for reducing the level of bed bug infestation. However, one limitation of pitfall traps is the collection of dust and debris, which allows bed bugs to escape from the smooth inside wall (Wang and Cooper 2011). Additionally, most products have been tested only for the common bed bug, so more studies with the tropical bed bug are urgently needed to test trap efficacy (Doggett 2012, Bed Bug Foundation 2016).

2.5 Bed bug management

From the early days of bed bug infestation, numerous eradication efforts have been tried, including natural enemies (Anonymous 1920, Millard 1932), physical (non-chemical) barriers (Bogdandy 1927), and various chemical methods (i.e., fumigation and spraying) (Rucker 1912). The most efficient method has proven to be correcting human behavior (to prevent bed bug infestation from outside sources) and teaching people to identify the early signs of infestation. This is as true today as it was a century ago. Long ago, various chemicals, including mercury, were applied to eradicate bed bugs, but the effects were short lived. As bed bugs rapidly dispersed as the human population, density, and mobility increased, bed bug infestations became widespread and quite serious. The development of central heating systems in the early 1900s aided bed bug dispersal over wider ranges in the world. Many people who suffered from bites and lack of sleep wanted to eradicate all bed bugs immediately, and some even used very toxic agents such as hydrogen cyanide gas. This compound is extremely dangerous to handle, and many people died. Nevertheless, it was sold at drug stores in the early 1900s. It later was replaced with sulfur gas, which is safer, but its efficacy was not great enough to eradicate bed bugs in a single treatment. Additionally, it has

weak residual performance, so infested places had to be treated several times (Marlatt 1916, Creel and Faget 1916).

Another approach to eradicating bed bugs is the use of common household pests such as ants and cockroaches as natural enemies. According to experiment fulfilled by Marlatt (1916), Pharaoh ants (*Monomorium pharaonis*) showed promising results. The workers carried all bed bugs away within a night. Another experimentation revealed that if the ant colony contained many eggs, the workers would catch more bed bugs to feed the ant larvae (Jameson 1933). However, cockroaches could not be a natural enemy since they did not eat the bed bugs (Johnson and Mellanby 1939).

In the 1940s, the pesticide DDT (Dechloro-diphenyl trichloroethane) was introduced to solve the bed bug problem perfectly (McGovran et al. 1944). It was well known that anybody could use the product and it was safe enough to apply on beds and even on the human body. DDT was applied in liquid or dust form to every corner of a house and even on human hair and the groin to eradicate bed bugs, lice, and fleas. It resulted in complete and immediate mortality of all household pests, and its residual effects lasted up to 3 years (Potter 2011, Usinger 1966). Prior to the 1950s, DDT had eradicated most of the bed bugs in the world, and bed bug infestations were not reported until their resurgence in the 1990s.

As the DDT (organochlorine) stated to show resistant reactions against bed bugs in 1947 (Johnson and Hill 1948), it was replaced to other members of the DDT family of Organochlorine insecticides such as lindane or chlordane. Alternatively, (next generations or other families) such as organophosphates (e.g. malathion, diazinon or dichlorvos), carbamates (e.g. bendiocarb, propoxur) (Cooper 2011). However, all the

Chlorinated hydrocarbon insecticides (organochlorides, organophosphates and cabamates) are banned (Romero et al. 2007, Davies et al. 2012).

According to Potter (2011), 99% of pest management professionals still rely on insecticides. However, the modern bed bugs developed high resistance level against Pyrethroids (Zhu et al. 2010, Cooper 2011) which have approved indoor use (Romero et al. 2007). Recently, additional resistant reaction was confirmed against Neonicotinoid (e.g. imidacloprid, thiamethoxam, acetamiprid and dinotefuran) (Romero and Anderson 2016) which was suspected by Haynes and Potter (2013). More recently, the resistance confirmed against Pyrrole (e.g. Chlorfenapyr) (Ashbrook et al. 2017). As both neonicotinoid and pyrrole have different mode of actions with pyrethroids, we can consider that the bed bugs are keep developing the resistances against various chemicals they have faced.

Most experts agree that management approaches rely too heavily on chemical treatment and that our mind set and behavior need to be refreshed. As in the era before DDT, alternative methods are being developed. The most commonly applied non-chemical method in the field is vacuuming. To reduce the allergic reaction caused by bed bug secretions, high-efficiency particulate air (HEPA) filters are highly recommended (Bérenger et al. 2015). If the vacuum head has a brush, the operator should be careful to avoid retaining bed bugs or eggs in the brush. At the same time, attachments can be used to scratch the surface gently to detach cemented eggs and bed bugs that are firmly holding onto the surfaces, which then can be removed by vacuuming.

Another treatment method involves extreme heat that is beyond the insects' biological limitations. A steaming machine should produce high-temperature dry

steam, which is much better than wet steam. The surface should be treated with steam slowly enough and with constant temperature in order to kill the bed bugs. As steam has limitations around heat-sensitive materials such as electronics, plastics, and painted surfaces, mixed treatment with a chemical application is recommended. Turning up the heat inside to 60°C for several hours is also commonly used in the United States and the European Union, it is expensive. In addition, if areas in the room experience lower temperature so that bed bugs survive after heat treatment, re-infestation can occur. In addition, bed bugs can reenter the premises by hitchhiking on items brought into the dwelling. In this scenario, minimized chemical treatment is also recommended to compensate for the limitations of non-chemical eradication methods (Davies et al. 2012). Mattress encasement is commonly used in the United States to kill bed bugs and to prevent them from infesting a mattress. It prevents bed bugs from entering or leaving the mattress. To achieve satisfying results, a properly manufactured product must be chosen. First, it must not have gaps between zippers. Second, the surfaces between the bed frame and the covered mattress should be smooth to avoid damaging the cover. Lastly, the cover material should be bed bug bite proof. Treating an infested mattress chemically before covering it is recommended, but it is difficult for chemicals to penetrate deep inside of the mattress. Another benefit of encasement is that it provides no hiding places for bed bugs, and the bright colored material often used makes it easy to detect the early stage of a bed bug infestation.

As modern bed bugs show high levels of pesticide resistance, desiccant dust such as diatomaceous earth or silica gel can be applied as an alternative (Davies et al. 2012). They work via a totally different mechanism than that of pesticides, so they may overcome the resistance issues (Romero et al. 2009). Dust are safe to human beings, they can be spread in every corner of an infested room (Miller and Rogers

2015), and they remain in place for a long period of time. Wild animals take a soil shower by digging in the ground and splashing the soil on their body or feathers (e.g., chickens) to detach mites or kill them by desiccation (Murillo and Mullens 2016). In the same way, if bed bugs contact the dust, their cuticle will be damaged and they will dehydrate within 2–3 days. However, a recent study reported that bed bugs developed resistance against desiccant dust by thickening their cuticle, which delayed dehydration up to 7–10 days (Lilly et al. 2016).

To achieve successful bed bug eradication, both the pest management professional and the residents need to be involved. Residents need to know how to identify evidence of a bed bug infestation, such as blood stains, empty skins, dead bodies, or feces (Doggett 2013b). They should carefully inspect luggage and second-hand furniture before bringing it into the house. In England and Sweden in 1930s, there were cleansing stations for house moving to treatment the bed bug infestation before moved in (Ministry of Health 1934).

Additionally, the newly moved in tenants were educated household insect's behaviors by government officers to prevent it (so called, Glasgow system) (Ministry of Health 1934, Hartnack 1939). As the Glasgow invested educating bed bug biology and behavior along with hygiene to their residents a century ago, various education programs are available both on and off line (e.g., web sites, webinars, schools, and hotels). More regulations, such as stopping the sale of the second-hand mattress or passing through treatment stations before moving in and out of states, will be needed. If officers of the modern government were able to visit and inspect newly moved-in residences, as was the case a century ago in Glasgow, dispersal of modern bed bugs would be reduced.

CHAPTER THREE

EVALUATION OF AN ARTIFICIAL FEEDING SYSTEM FOR LABORATORY CULTURE OF THE TROPICAL BED BUG *Cimex hemipterus* (F.) AND THE COMMON BED BUG *Cimex lectularius* (L.)[1]

3.1 Introduction

Bed bugs are hematophagous, thus all nymphal stages and adults require blood meals to moult out, mate, and produce eggs. This requirement of providing regular blood meals makes it difficult for researchers to culture and enlarge bed bug populations needed for research. According to Aak and Rukke (2014), less than half of researchers artificially feed test populations of bed bugs, whereas the remainder naturally feed them using animal or human host body parts. Historically, most bed bug researchers fed bed bugs using live animals such as rabbits (United States Department of Agriculture 1963, Wattal and Kalra 1961), rats, chickens, guinea pigs, bats, or human hosts. However, natural feeding has limitations when large populations of bed bugs are needed. In particular, natural feeding can cause serious allergic reactions in human hosts (Minocha et al. 2017) and even death of animal hosts.

Numerous reports have been published about artificial feeding methods for blood sucking arthropods such as mosquitoes (Deng et al. 2012), lice (Yoon et al. 2006, Takano Lee et al. 2003a, 2003b), and bed bugs (Montes et al. 2002, Chin-Heady et al. 2013, Aak and Rukke 2014). Most of these studies were based on the understanding

[1] Submitted to the *Journal of Economic Entomology*.