ASSESSMENT ON INSECTICIDE RESISTANCE IN BED BUGS (*CIMEX LECTULARIUS L*) COLLECTED FROM A POULTRY FARM

by

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Dedicated to my family and friends. Without them, I wouldn't be here today.

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ABSTRACT

An emerging issue in North America and other developed countries around the world is dealing with the blood feeding ectoparasite, *Cimex lectualrius* L. Bed bugs not only infest human homes but also poultry (chicken) farms, in particular cage free barns. In comparison to conventional caged housing systems, cage free facilities offer more hiding places for bed bugs. As such, with the increase in the number cage free poultry facilities across the USA, there is an increased risk that bed bug infestations could become more frequent in chicken houses. To combat these infestations new research is needed to help understand insecticide resistance displayed by poultry house bed bugs in comparison to a susceptible bed bug population. In addition to insecticide resistance, the efficacy of insecticides used in poultry houses could be affected by dust and chicken manure that contaminate all areas of a barn. The objectives of this study were to:

I. To determine the status of insecticide resistance in the PH2019 (poultry house 2019) bed bug strain compared to the laboratory strain.
II. To analyze the effects of substrate contamination on insecticide efficacy against the PH2019 bed bug strain.

This study included experiments to test resistance of four poultry house registered products (Talstar, Tempo SC, EcoRaider, and Rabon) in the PH2019 poultry bed bug population in comparison to the laboratory strain in direct spray and residual bioassays. Additionally, experiments to test the efficacy of the above-mentioned products against the PH2019 field strain on contaminated and clean stainless-steel tiles were also performed. Data from resistance assessment direct spray and residual bioassays suggested that the PH2019 population was not resistant to the two pyrethroid insecticides (Talstar and Tempo SC) and the essential oil product EcoRaider. However, the PH2019 strain displayed >100 and 800-fold resistance to the

organophosphate insecticide Rabon (tetrachlorvinphos) in direct spray and residual bioassays, respectively. Contamination of the bioassay substrate with chicken manure did not reduce insecticide efficacy against the PH2019 population.

In conclusion, this study suggests that the efficacy of certain pyrethroid products is superior to that of essential oil-based and organophosphate products dismissing the suggestion that all bed bugs collected after 1990's exhibit cross-resistance to pyrethroid insecticides. However, the data does suggest the presence of organophosphate resistance in the PH2019 strain. Finally, contamination of the bioassay substrate did not change the outcomes of the bioassays and product efficacy was statistically equivalent on contaminated and clean tiles.

CHAPTER 1. LITERATURE REVIEW AND OBJECTIVES

1.1 Biology of the Bed bug

Bed bugs, bat bugs, and swallow bugs are all common names for different insect species that belong to the family Cimicidae of the order Hemiptera. All are hematophagous and primarily feed on humans, bats, and birds (Krinsky, 2019). The most common species of bed bugs in North America is *Cimex lectularius* L, which is known to primarily feed off human and poultry hosts. An adult bed bug can fully engorge on a blood meal between 10 to 20 minutes (Reinhardt & Siva-Jothy, 2007) if not interrupted. The bed bug life cycle is dependent on blood meals as it is the only nutrition and hydration support bed bugs receive (Usinger, 1966). All juvenile stages require a blood meal prior to molting and for the adults to produce sperm and eggs (Usinger 1966, Baker 2020). Feeding frequency is dependent on three main factors: digestion rate, environmental temperature, and host availability (Reinhardt and Siva-Jothy, 2007). The distinguishing features of *C. lectularius* L. are that they dorsoventrally flattened, oval shaped, mahogany colored with slender pronotum and a wider head. Wings are never present and on average adults are 5 mm in length (Krinsky, 2019). The presence of a paragenital sinus on the fifth abdominal sternite indicates a male (Krinsky, 2019). The paragenital sinus allows the male to insert it's aedeagus to fertilize the female through a process called traumatic insemination (Krinsky, 2019). This process requires the male to mount the female (Reinhardt and Siva-Jothy, 2007). Once mounted the male immediately begins probing the female's abdomen until he reaches the site of penetration called the ectospermalege (Davis, 1956). In females, the majority of the male sperm is stored within the paragenital system, an area called the seminal conceptacles (Carayon, 1966). Sperm migrates to the ovaries for egg fertilization right before oviposition (Reinhardt and Siva-Jothy, 2007), however, egg production can rapidly stop due to lack of blood meals (Davis, 1956). The negative

consequence of traumatic insemination is reduced fitness for females (Mellanby, 1939) due to repeated damage to the abdominal wall and sometimes rupture of the gut (Kemper, 1933).

Egg production starts two to five days after a blood meal and mating, this production slows down once a female is 30 to 200 days old (Doggett et al. 2018). Female bed bugs (C. *lectularius*) will lay 5-8 eggs per week for 18 weeks if conditions are optimal (Doggett et al. 2012). The eggs are 1mm in length and cream in color are cemented to rough surfaces and hatch within 9 to 12 days under optimal conditions (Doggett et al. 2012). The immature life stage includes 5 juvenile instars before the final molt into an adult (Doggett et al. 2018). Host availability is a prominent factor in the life cycle of a bed bug, which can determine the egg to adult development time and subsequent reproduction. Additionally, the life span of bed bugs is highly variable due to environmental conditions and temperatures. In the average home or hotel living conditions and temperatures, bed bugs have a 2-month growing period and an adult can live approximately 4 to 12 months in addition to that (Busvine 1980, Maschek 2015).

Bed bugs are ectoparasites, meaning they feed externally on the host. Bed bugs usually leave their refugia to locate a host or seek a blood meal, this practice poses a high risk of mortality due to the amount of outside exposure (Doggett et al. 2018). After finding a host and feeding to repletion they return to their harborage, there is a positive effect on the feeding of first instar nymphs when fed adult bed bugs return from the host (Balvín et al. 2019). Studies have suggested bed bugs find their hosts using multiple factors such as heat, CO_2, and body odors (Gaire et al. 2020). Bed bug antennae are crucial for sensing thermal cues based on nutritional status (Berry, 2021) however, these cues seem to be limited to short distances (Gaire et al. 2020).

1.2 Resurgence of bed bugs in human dwellings and other institutions

In the early 1990's bed bug infestations were commonplace. Almost every home in North America had experienced a bed bug infestation during that period and their sightings in poultry houses had been recorded across the Southwest and Southeast United States (Kulash, 1946). In 1945, a *C. lectularius* infestation was found on a large poultry farm in North Carolina (Kulash, 1946), which had been established for more than fifteen years. By 1958, bed bugs were mostly thought to be eradicated from North America (Usinger, 1966) mainly through the use of DDT and other synthetic insecticides. In the late 1990s, the bed bug resurfaced back into households and poultry barns. Resurgence first started in major metropolitan cities and eventually branched out across North America. There were two species of bed bugs that resurged in the late 1990's, *C. lectularius* and *C. hemipterus* (the tropical bed bug) both as major urban pests (Ashbrook et al. 2017). C. *lectularius* L became globally widespread during the resurgence, while C. *hemipterus* was more limited to 30° northern and southern latitudes (Ashbrook et al. 2017).

Due to the unexpected resurgence that happened 40 years after the near eradication of bed bugs from North America, pest management companies lacked experience and knowledge to combat these new infestations (Doggett et al. 2018). Not only did the resurgence cause an issue for the pest control companies, but it also started the controversial topic on liability and property management for the rental and hotel industry. As of 2020, the bed bug resurgence is still a growing global issue (Reinhardt and Siva-Jothy 2007, Doggett et al. 2018). Global resurgence is believed to be largely caused by the development of insecticide resistance to insecticides (Romero et al. 2007, Dang et al. 2017), increased international travel (Alalawi, 2014) and increase in the exchange of second-hand furniture.

1.3 Medical and economic significance of bed bugs

When an infestation occurs in human dwellings, there is a possibility of medical complications associated with bed bug bites. Individuals may display symptoms of hypersensitivity, allergic reactions or in extreme cases experience breathing difficulties, and often these symptoms are more severe if an individual has underlying health conditions such as asthma (Doggett et al. 2018). An individual can experience significant blood loss if they have a severe infestation in their home (Doggett et al. 2018). Lowered hemoglobin levels and fatigue are symptoms of blood loss due to bed bug bites (Doggett et al. 2018). Infestations in poultry houses can cause irritation and blood loss on avian hosts (Mullens and Murillo, 2017). Feeding wounds can place the host at risk for a secondary infection, yet these are scarcely documented (Reinhardt and Siva-Jothy, 2007). Sleep loss and anxiety are common consequences of living in bed bug infested dwellings, these symptoms are caused by the presence bites or the just the mere knowledge or phobia associated with the infestation (Doggett et al. 2018).

Misuse of chemicals to treat an infestation can also cause serious harm to the individual(s) or the environment, and the risks associated with chemical exposure could be life threatening (Doggett et al. 2018). Bed bug infestations can be found inside of medical and emergency facilities making it difficult for communities to receive risk free health care resources (Doggett et al. 2018). Bed bugs are able to carry several different infectious human pathogens in their guts, however, currently they are not known to transmit any of these pathogens to humans through blood feeding (Reinhardt and Siva-Jothy, 2007).

Economic impacts come from organizations failing to proactively respond to the bed bug infestations and pest management associated damages continue to grow not only in the hospitality industry, private and communal households but also inside poultry houses (Reinhardt and Siva-Jothy, 2007, Doggett et al. 2018). Some impacts that may affect the poultry industry are costs of

bed bug control measures, extra labor required for deploying bed bug control , profit loss from egg shells being stained with bed bug fecal matter, risk of employees bringing infestations home, decrease in egg production, causing birds to suffer from anemia and possibility of avian death (Reinhardt and Siva-Jothy 2007, Axtell 1999, Cater et al. 2011, Doggett et al. 2018). Infestations in poultry houses cause irritation and blood loss to birds, but again pose no threats in spreading diseases (Mullens and Murillo, 2017). Parasite management options vary depending on several factors: bird age, hen condition and housing type (Mullens and Murillo, 2017). Although the cage-free housing system has benefits in reducing some pests such as flies, the structures tend to have many cracks and crevices for ectoparasites to hide during the day (Mullens and Murillo, 2017). Currently, the main priorities in integrated poultry pest management are (i) determining bed bug damage thresholds in poultry systems and (ii) investigating the relationship between monitoring metrics and effectiveness of the control measures (Mullens and Murillo, 2017). The economic issues that cage-free egg production companies face due to bed bug infestations are complex. Two examples of these issues are worker safety as bed bugs can bite and be transported by employees, reduction in egg value due to bed bug fecal spots on shells, and costs of pest management (Reinhardt and Siva-Jothy, 2007, Doggett et al. 2018).

1.4 Bed Bug Control and Insecticide Resistance

In human dwellings, multiple control techniques are used to manage bed bugs (Bennett et al. 2016). Including non-chemical options such as exclusion, physical removal, and the creation of adverse environments via heat treatments. Preventing introductions or exclusion of the bed bugs from poultry barns should be the initial goal, especially if another poultry house on the property or in the vicinity is already infested (Axtell and Arends, 1990). Chemical formulation options for the management of bed bugs include the use of sprays, dusts, and fumigants. The insecticide

classes registered globally for bed bug control are pyrethroids, organophosphates, carbamates, neonicotinoids, halogenated pyrroles, insect growth regulators, inorganics, mineral compounds, and botanical insecticides (United States EPA, 2017). However, in cage-free poultry house systems, the number of insecticide products registered for use is low (Mullins and Murillo, 2017). Botanical insecticides such as essential oils are the preferred pest management tools because they are relatively low risk to birds and humans. Pyrethroids, synthetic pyrethroids, organophosphates, carbamates, and botanical insecticides are being used in poultry house bed bug management (Tabler et al. 2018). Currently, there is little information available on the efficacy of these insecticides against bed bugs in poultry house systems (Mullins and Murillo, 2017). Also, little is known about the status of resistance that poultry house bed bugs possess to insecticides used for their control.

Insecticide resistance is a 'heritable change' in the sensitivity of a population, which is evident from repeated failure of an insecticide product to achieve the expected level of control when used according to the label recommendations (Insecticide Resistance Action Committee, 2020). Misuse or overuse of a product can influence the evolution of insecticide resistance. As described in the previous section, one of the factors responsible for the resurgence of bed bugs is their initial development of resistance to the widely used organochlorine insecticides especially DDT in the 1950s, which conferred cross-resistance to pyrethroid class insecticides that were predominantly used for indoor pest control in the 1990's (Romero et al. 2007, Dang et al. 2017). Knockdown resistance (kdr-type) involves reduced target site sensitivity caused by mutations (Dong et al. 2014). Cross-resistance occurs when the resistance to one insecticide confers resistance to another pesticide from the same class, even if a population has not been exposed to the later product (Insecticide Resistance Action Committee, 2020). The target site for both

pyrethroid insecticides and DDT is the voltage-sensitive sodium channel (Soderlund and Bloomquist 1990, Punchihewa et al. 2019), where membrane proteins are essential for the initiation and propagation of the action potential in neurons or other excitable cells (Dong et al. 2014). More than 50 sodium channel mutations have been linked with kdr-like resistance to pyrethroids in different insect species including bed bugs (Dong et al. 2014). Another mode of pyrethroid resistance is reduced cuticle permeability. Reduced penetration can increase the chances of cross-resistance in bed bugs (Lanning et al. 1996, Ahmad et al. 2006, Zhu et al. 2013). Several populations of *C. lectularius* in North America have developed resistance to pyrethroids, various neonicotinoids, and exhibit reduced susceptibility to chlorfenapyr, a pyrrole class insecticide (Romero et al. 2007, Zhu et al. 2013, Romero and Anderson 2016, Ashbrook et al. 2017).

Bed bug infestations can be difficult to manage inside poultry houses due to label restrictions and harborage locations. It can be easier to treat and control an infestation after the poultry are removed (Tabler et al. 2018). Pyrethroid insecticide products such as Oxy-Fly (Lambda-cyhalothrin), Tempo 20WP (beta-cyfluthrin), and Tempo SC Ultra (beta-cyfluthrin) are insecticides that can only be used to treat houses when birds are not present (Tabler et al. 2018). Additionally, more research is still needed to decipher the status of insecticide resistance in poultry house bed bug strains to various insecticides (Steelman et al. 2009). It is also important to determine residual efficacy of various insecticide products applied to porous (e.g., wood) and non-porous (e.g., metal or plastic) because bed bugs in cage-free poultry houses may only encounter dry chemical residues, which could be less efficacious in comparison to directly sprayed insecticides. It is important to identify the most effective insecticides, application techniques and

control measures for bed bug management in cage-free poultry houses as the poultry industry slowly transitions to using cage-free housing systems for egg and broiler production.

1.5 Cage-Free Poultry Production

The poultry industry has changed in recent years to consider better standards of animal welfare. Judged based on three scientific principles: ability to cope with the environment while having the needs met, an animal's subjective experience, and the ability to perform natural behaviors (Hartcher and Jones, 2017). Due to these considerations of animal welfare, large corporations such as McDonald's and the retail grocery store industry have pledged to use and sell only cage-free eggs in the near future.

The cage-free housing systems for poultry can extend their range of behavioral expression, space, hygiene, and production efficiency (Hartcher and Jones, 2017). To provide proper welfare to egg laying hens, a producer must consider the following: skeletal health, disease, severe feather loss due to pecking at one another, cannibalism, movement, perching, nesting, dustbathing, and the ability to forage or explore (Hartcher and Jones, 2017). The main advantages to cage-free housing systems are low rates of infectious disease transmission and a decrease in severe feather pecking. Adoption of cage-free versus caged housing is a topic of debate among producers because both are efficient regarding food safety and egg quality (Holt et al. 2011). However, cage-free systems have the potential to fulfill the welfare requirements that the other housing systems lack. The skeletal health issue could be combatted by better planning and design (Hartcher and Jones, 2017). In cage-free housing systems producers have encountered both endoparasites and ectoparasites such as bed bugs. Bed bugs are considered a temporary parasite because they do not live directly on the bird but still routinely use it as a food source. Temporary parasites are some of the worst threats to laying hens in cage-free housing systems (Mullens and Murillo, 2017). Poultry

house design helps decide the severity of parasites and creates an environment that can influence pest complexes (Mullens and Murillo, 2017). Other practices such as beak trimming, to prevent pecking or cannibalism, can make flocks or individuals more susceptible to bed bugs due to their inability to dislodge these temporary parasites from their body (Mullens and Murillo, 2017). Limited pest control options are available for use inside poultry houses, and even less options for organic egg production. Nontraditional pesticides that are used in organic systems are often not well tested, which makes parasite control particularly challenging (Mullens and Murillo, 2017). An effective practice in the biosecurity of a flock, is to disinfect the entire housing system after depopulating and before bringing in a new population of birds (Mullens and Murillo, 2017). This can help reduce the risk of parasite introduction to a new flock. In many scenarios, practicing management of the infestation makes more economical sense (Mullens and Murillo, 2017) than trying to eradicate all the parasites. Certain synthetic and botanical insecticides can be used in poultry houses to control bed bugs and other insect pests but continued testing for resistance is crucial due to the ability of bed bugs to develop resistance to many different chemicals. Additionally, as explained in previous sections, bed bugs have already developed resistance to certain insecticide classes (e.g., pyrethroids and organophosphates) that are registered for use in poultry.

1.6 Rationale and Objectives

Insecticide treatments are the main tool for the management of bed bug infestations in cage-free poultry facilities. Bed bugs are known to develop resistance to insecticides, through various mechanisms such as target-site insensitivity, increased activity of detoxification enzymes and reduced cuticular penetration (Romero et al. 2007, Zhu et al. 2013, Dong et al. 2014). Studies focused on determining resistance status to various chemicals including botanicals can help refine

strategies to reduce the risk of resistance development through identification of efficacious insecticides that can be used in rotation with other non-chemical and preventative control measures. Several studies have tested commercially available insecticides on poultry bed bugs. Although many bed bug populations have shown the potential to develop resistance to the organophosphate insecticide, chlorpyrifos, it is still effective against poultry bed bugs collected from Mississippi (Goddard and Maschek, 2015). Chlorpyrifos is only certified for use in poultry houses, while the hens are not present (Goddard and Maschek, 2015). Another study was conducted using glass vial bioassays and tracked susceptibility of DDT resistant colonies to twelve different insecticides (Steelman et al. 2009). None of the previous studies have compared the residual efficacy of formulated pesticide products on non-porous (metal) substrates that are commonly found in cage-free poultry houses. To address the mentioned knowledge gaps, the of this research is designed to:

I. To determine status of insecticide resistance in the PH2019 bed bug strain relative to the susceptible laboratory strain.

II. To analyze the effects of substrate contamination on insecticide efficacy against the PH2019 strain bed bugs.

CHAPTER 2. POULTRY HOUSE BED BUG POPULATIONS DO NOT EXHIBIT PYRETHROID RESISTANCE

2.1 Introduction

Bed bugs (*Cimex lectularius*) are small, flat, oval-shaped shaped and wingless insects belonging to order Hemiptera and family Cimicidae that feed off the blood of hosts including humans, birds, and bats (Reinhardt and Siva-Jothy 2007, Maschek 2015).

Since the early 1990's, bed bug infestations have been reported in poultry barns (Kulash, 1946). After widespread exposure to organochlorides in the 1940's their presence dwindled. Although bed bug infestations can get established in caged as well as cage-free chicken housing systems, cage-free systems offer a more conducive environment in which bed bugs can reside. Harborage areas that are not found in caged systems include chicken perches, nesting boxes, areas around nest sites, and extensive access to the barn floor (Mullens and Murillo 2017, Machtinger and Martin 2021). As the industry converts from battery cages to cage-free housing, due to reasons including animal welfare (Hartcher and Jones, 2017), bed bug infestations in poultry housing are becoming more frequent (Axtell and Arends 1990, Mullens and Murillo 2017). With increased bed bug infestations in cage-free poultry facilities, there are concerns that they may be transferred to other locations in the vicinity of the poultry barns by hitchhiking on clothing, shoes of workers (Maschek, 2015). From the perspective of chicken welfare and productivity, bed bugs have been implicated in 10% drop in productivity in egg production (Cater et al. 2011, Maschek 2015). Synthetic pesticides are one of the important tools used for bed bug control in poultry houses. However, due to the large size of poultry barns and complexity of cage-free housing systems, which provide plenty of hiding spots for bed bugs, it is difficult to thoroughly treat a poultry barn with pesticides particularly when chickens are present (Steelman et al. 2009). Because of these

factors, achieving complete (100%) control of bed bugs with pesticides is very difficult (Mullens and Murillo, 2017). Additionally, insecticide resistance, which allows bed bugs to withstand pesticide exposure through various mechanisms such as target-site insensitivity, increased activity of detoxification enzymes and reduced cuticular penetration (Romero et al. 2007, Adelman et al. 2011, Zhu et al. 2013, Ashbrook et al. 2017, Gaire et al. 2020, 2021), are other hurdles limiting the effective control of bed bugs. While the efficacy of commercially available insecticides against at least two bed bug populations collected from infested poultry barns has been previously determined (Steelman et al. 2009, Goddard and Maschek 2015), only one study (Ashbrook et al. 2017) has measured comparative efficacy of insecticides using both a laboratory susceptible and a field collected poultry house population from Tennessee (USA). The unavailability of comparative insecticide efficacy data between laboratory susceptible and field collected populations is a limiting factor in estimating the scope and extent of the resistance problems in poultry house bed bug populations. To bridge this knowledge gap, the objectives of this study were to determine the effectiveness of three currently used synthetic insecticides and one essential oil product against laboratory susceptible and poultry house bed bug populations by conducting (i) direct spray and (ii) residual bioassays on a non-porous stainless-steel substrate.

2.2 Materials and Methods

2.2.1 Bed bug Strains

Two strains of *C. lectularius* were used, the susceptible Harlan laboratory population which was used as an insecticide susceptible baseline strain (Ashbrook et al. 2017) and the field collected Poultry House 2019 (PH2019) strain. The PH2019 strain was collected from a cage-free layer hen facility in the Midwest region in June 2019. Since its collection from the field in 2019, it has been

lab-adapted and is reared in the laboratory at Purdue University. Both populations were maintained at 27 ± 1°C, 50 ± 10% RH and a 12:12h (L:D) cycle in a temperature controlled environmental chamber (Percival Scientific, Perry, IA). Both populations were fed on defibrinated rabbit blood purchased from Hemostat Laboratories in Dixon, CA. The parafilm membrane feeding method (Chin-Heady et al. 2013) was used to feed rabbit blood to bed bugs. Both bed bug populations were fed 3–5 days prior to their use in bioassays. The average weight of the of Harlan females was 0.053 g (± 0.001 g) and for Harlan males it was 0.042 g (± 0.0007 g). The average weight of the of PH2019 females was 0.056 g (± 0.001 g) and for PH2019 males it was 0.042 g (± 0.0006 g).

2.2.2 Chemicals

Synthetic insecticide products used in this study are labelled for use inside poultry houses. These synthetic insecticide products include: Talstar® Professional (Bifenthrin 7.9%, other ingredients 92.1%), Tempo® SC (ß-Cyfluthrin, Cyano(4-fluoro-3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylate 11.8%, other ingredients 88.2%) and Rabon® (Tetrachlorvinphos 50%, other ingredients 50%). Talstar and Tempo SC are synthetic pyrethroids, Rabon is an organophosphate. In addition to synthetic insecticides, EcoRaider®, an essential oil-based product, was also tested because it shows efficacy against insecticide resistant bed bugs (Gaire et al. 2019, 2021). Except EcoRaider, which was a ready-to-use formulation, all insecticides were diluted in tap water as per their label-recommended dilution rates. (Talstar was tested at a concentration of 0.06%, whereas Tempo SC with a and Rabon were tested at concentration of 0.05% and 1.0%, respectively.)

2.2.3 Direct Spray Bioassays

1/16th inch thick stainless-steel tiles that were cut to desired dimensions (4 x 4 inches) by the Purdue University Fabrication Workshop to simulate some of the substrates the bed bugs in poultry houses encounter in a cage-free barn. Each bioassay replicate representing a single stainless-steel tile included 20 bed bug adults (males and females in 1:1 ratio). Bed bugs were confined on the stainless-steel tile by using an open-ended base or bottom part of a 100 x 15 mm plastic Petri dish (Fisher Scientific, Waltham, MA) as shown in Fig. 2.1. The open-ended Petri dish base was affixed to the tile using glue (Elmer's, Westerville, OH). Insecticide application volume was equivalent to the standard rate of 1 gallon per 1000 square feet, which translated to 0.42 mL of water-diluted or ready to use insecticide or essential oil product for each 4 x 4-inch tile. This amount was administered using a 60 mL spray bottle (Meijer, Grand Rapids, MI) (Fig. 2.2).

The number of spray pumps required to dispense 0.42 mL volume of insecticide on to the tile was pre-determined. Additionally, the spray bottle was weighed before and after spraying each tile to ensure that desired amount of insecticide was applied on bed bugs/ tiles. While spraying the insecticide on to the bed bugs, the spray bottle was held at a distance of 5–10 cm from the insects or substrate (Fig. 2.3). After spray treatment, bed bugs were held on the steel tiles for five minutes. After 5 minutes had elapsed, they were transferred to a filter paper (Whatman #1.55 mm) lined 60 x 15 mm Petri dish (Falcon, Hainesport, NJ) (Fig. 2.4). Control bed bugs were sprayed with 0.42 mL of distilled water and were subsequently processed using procedures described above for different insecticides. Each pesticide and control treatment consisted of five replicates. Post direct spray treatment, Petri dishes containing bed bugs were held in temperature (27 $\pm$ 1°C) controlled environmental chambers that were used for rearing bed bugs. Mortality was recorded at 5 minutes,

30 minutes, 4 hours, 1 day, 3 days, 5 days, and 7 days. Bed bugs showing immobility when prodded with a pair of forceps.

2.2.4 Residual Bioassays

Bioassays with dried residues of three synthetic insecticides and one essential oil product were also conducted on custom made 4 x 4-inch stainless steel tiles affixed with a plastic Petri dish base (Fig. 2.1). As with direct application bioassays, all chemicals except Eco Raider (ready to use product) were diluted in distilled water as per their recommended label rate for use in poultry barns. A hand sprayer was used to treat tiles with 0.42 mL of the insecticide solution. Control tiles were treated with 0.42 mL of tap water. After treatment, insecticide and water-treated tiles were allowed to dry overnight (16–24 h) at room temperature (21–22°C). After the tiles were dry, 20 adult bed bugs (1:1 ratio of males: females) were released and maintained on the tiles for 4 hours. The plastic Petri dish ring prevented bed bugs from escaping the insecticide or water-treated area of the tile (Fig. 2.1). After the 4-hour exposure on treated or control tiles, bed bugs were transferred to Whatman filter paper lined 60 x 15 mm plastic Petri dishes (Falcon) in Fig. 2.4. Five replicates with 20 adult bed bugs each were conducted for each insecticide and control treatment. Petri dishes were then placed in an environmental chamber that was used for rearing. Mortality recording started while the bed bugs were confined to the tiles (30 minutes, 1 hour and 4 hours). Subsequent mortality observations were recorded at 1 day, 3 days, 5 days, and 7 days.

2.2.5 Data analysis

Cumulative mortality data (i.e., number dead at various time points) from direct spray and residual bioassays was subject to probit analysis in SAS 9.4 to determine median lethal time (LT_{50}) values for both Harlan and PH2019 populations. In instances where the mortality data was

heterogenous the covariance matrix was multiplied by a heterogeneity factor (HF). For resistance ratio determinations at the LT50 level, the Harlan strain was used as the baseline population. Resistance ratios and their corresponding 95% confidence intervals (CIs) were calculated by using the method described by Robertson et al. 2017. Ratios were considered significant if their 95% CIs did not overlap with the value one (Robertson et al. 2017).

2.3 Results

2.3.1 Comparative Efficacy of Insecticides Against the Susceptible and Poultry House Strains in Direct Spray Bioassays

Average mortality in control treatments not exposed to insecticides ranged from 0% to 9% during the 7-day experiment. Time-mortality or LT_{50} data for EcoRaider, Tempo SC, Rabon, and Talstar generated using the Harlan and PH2019 strains are presented in Table 1. The LT_{50} (median lethal time) estimates for most of the products were generally lower for the Harlan strain when compared to the PH2019 strain except for Tempo SC (Table 1.1). The resulting LT_{50} resistance ratios (RR_{50}) determined for the PH 2019 population in increasing order of their magnitude were as follows: 0.12-fold for Tempo SC, 10.97-fold for EcoRaider, 14.59-fold for Talstar and 119.70-fold for Rabon. The resistance ratios exhibited by the PH2019 strain to EcoRaider, Talstar and Rabon, were statistically significant based on non-overlap of 95% confidence intervals (Table 2.1). In contrast, the 8-fold higher susceptibility (0.12-fold resistance) of the PH2019 strain to Tempo SC in comparison to the Harlan population was not statistically significant (Table 2.1). These unexpected results for Tempo SC are likely caused by absence of mortality in the Harlan strain during initial observation intervals up to 0.167 d or 4 h, followed by 100% mortality at 1 d and beyond. Although the Tempo SC LT_{50} values for Harlan were higher in comparison to PH2019,

the LT_{90} values were almost identical (0.47 d for Harlan and 0.51 d for PH2019) thus indicating absence of resistance (data not shown).

When percent mortality data at the bioassay end point (i.e., 7 d) were considered, there was no statistically significant difference in mortality of Harlan and PH2019 populations for Talstar, Tempo SC and EcoRaider (Fig. 2.1; $P > 0.05$; student's t-test). Although, statistically significant difference in mortality of the two populations was not seen, it should be noted that complete (100%) mortality was never achieved for PH2019 (Fig. 2.5). However, mortality levels for Rabon differed significantly between the two populations (Fig. 2.5; $P<0.05$ at 0.0025, student's t-test).

2.3.2 Comparative Efficacy of Different Insecticides Against the Susceptible and Poultry House Strains in Residual Bioassays

Average control mortality ranged from 0% to 7%. As observed in direct spray bioassays, the LT_{50} values for all insecticides except Tempo SC were lower for the Harlan strain than for the PH2019 strain (Table 2.2). The LT_{50} resistance ratios for different insecticides displayed by the PH2019 strain in increasing order of their magnitude were as follows: 0.57 for Tempo SC, 1.83 for EcoRaider, 5.45 for Talstar and 878.78-fold for Rabon. (Table 2.2). While the LT_{50} values or corresponding resistance ratios for Rabon (>800-fold resistance) were statistically different between the PH2019 and Harlan, the LT estimates and resistance ratios calculated for Talstar and EcoRaider were not significantly different between the two bed bug populations. The 2-fold higher susceptibility or lower resistance of the PH2019 population to Tempo SC in comparison to Harlan was also statistically non-significant.

At the bioassay end point (7 days), statistically significant differences between Harlan and PH2019 existed only for Rabon (Fig. 2.6; $P<0.05$; student's t-test). Despite the absence of mortality differences between the two strains for Talstar, Tempo SC and EcoRaider, it is

interesting to note that 100% mortality of the PH2019 was never achieved at 7 days with these three insecticides (Fig. 2.6).

2.4 Discussion

Resistance continues to be defined as "the development of a strain capable of surviving exposure to a label recommended concentration of insecticide that is lethal to a majority of individuals in a normal population" (ffrench-Constant et al. 1990, Steelman et al. 2009). Global resurgence of the bed bug has been linked, in part, to the insecticide resistance bed bugs have developed to several pyrethroid class insecticides (Romero et al., 2007, Dang et al. 2017). Several populations of *C. lectularius* L. in human dwelling habitats have developed resistance to pyrethroids and neonicotinoids, and exhibit reduced susceptibility to chlorfenapyr, a pyrrole class insecticide (Romero et al. 2007, Zhu et al. 2013, Romero and Anderson 2016, Ashbrook et al. 2017, Gaire et al. 2019, 2021).

Unlike the studies conducted with bed bugs collected from human dwellings, this study did not find high levels of pyrethroid resistance in the PH2019 strain, which was collected from cage-free chicken barns. These are interesting results due to various reasons. First, due to the widespread use of DDT for bed bug control during the 1940's and 1930's, cross-resistance to pyrethroids, which like DDT also bind to the voltage-gated sodium channels (VGSC), is common in bed bug populations from North America (Zhu et al. 2010). Secondly, mutations in the VGSC gene that impart resistance to pyrethroids, and DDT have been detected in the PH2019 strain (Gondhalekar et al. unpublished data). Lastly, there is a history of pyrethroid insecticide use at the site where the PH2019 strain was collected. Therefore, the fact that PH2019 strain did not exhibit any resistance to Talstar (bifenthrin), and Tempo SC (cyfluthrin) is surprising. Nonetheless, when the available data for bed bug populations collected from poultry farms in North American is considered, the

absence of pyrethroid resistance in the PH2019 strain appears to follow a trend. In this regard, field collected bed bugs that were directly tested in the laboratory for susceptibility to various insecticides reported high efficacy of bifenthrin and B-cyfluthrin (Steelman et al. 2009, Goddard et al. 2013, Goddard and Maschek 2015). Similarly, in discriminating concentration bioassays conducted with bifenthrin (Talstar), another poultry house bed bug strain collected from the state of Tennessee, did not exhibit significantly reduced mortality when compared to the susceptible Harlan population (Ashbrook et al. 2017). Overall, the trend of bifenthrin and β-cyfluthrin efficacy and/or resistance observed in this, and previous studies suggests that pyrethroid resistance may not be a major issue affecting the control of poultry house bed bugs.

Certain essential-oil based products (e.g., EcoRaider) have shown promising results for controlling bed bugs collected from multifamily housing complexes (Singh et al. 2014). The same product was also found to be effective against a deltamethrin resistant bed bug population (Gaire et al. 2021). In the present study, the PH2019 population displayed ~10-fold resistance to EcoRaider in direct spray bioassays, but in residual tests, the difference in LT_{50} values of Harlan and PH2019 was only 2-fold. Also, at 7 d, >95% mortality of the PH2019 bed bugs was observed in both bioassays. These results indicate higher efficacy of EcoRaider in comparison to another essential oil product (Eco Exempt), which killed <50% of the tested poultry house bed bugs after 24 h of continuous exposure (Goddard and Maschek, 2015).

Resistance to organophosphate (OP) class insecticides is not considered a problem in bed bug populations as only five countries, not including USA, have presented reports indicating OP resistance in bed bugs (Doggett et al. 2018). Insecticide bioassays could be used to detect insecticide resistance and mechanisms within bed bugs (Dang et al. 2017). Organophosphates have been linked to resistance in other orders of insects such as Diptera and Coleoptera (Hamm et al.

2006, Bohounton et al. 2021). Organophosphates are considered non-persistent within the environment and do not bioaccumulate in food; however, the USA has mostly phased out the use of OPs in residential areas due to the implementation of the Food Quality Protection Act of 1996. However, there are 2 OP insecticides that are permitted to be used inside the poultry barns. While one study that tested technical grade OP insecticides (diazinon and dichlorvos) against poultry housed bed bug reported low efficacy in one of the three total populations (Steelman et al. 2009) another reported high efficacy to products containing chlorpyriphos (Durasheild®) and a mixture of tetrachlorvinphos and dichlorvos (Ravap®). Resistance to tetrachlorvinphos at the LT_{50} level in the PH2019 strain varied between 100-fold in direct spray bioassays to >800-fold in residual bioassays. In congruence with high LT_{50} resistance ratios for Rabon, mortality observed in the PH2019 population at the 7d interval were 10% or less in both bioassays, whereas mortality approached 95–100% levels in the Harlan strain at the same time point. The label for Rabon does not list bed bugs as the target pest, but it was regularly used at the site from where PH2019 strain was collected, mainly for the control of flies and darkling beetles. To further investigate the extent of OP resistance in the PH2019 strain it will be important to conduct efficacy and/or resistance tests with Durashield and Ravap, which are restricted use products and require a pesticide applicator license for field or lab-based testing.

While extrapolating the results of this study and other previous reports to the control of bed bug infestations in poultry barns, two factors need to be taken into consideration. First, multiple studies have now confirmed high efficacy or absence of resistance to pyrethroid insecticides in poultry house bed bugs (Steelman et al. 2009, Goddard and Maschek 2015, Ashbrook et al. 2017). Given this trend, pyrethroid class insecticides appear promising for the control of poultry house bed bugs. With respect to essential oil-based and OP insecticides their efficacy and resistance

status is variable among different poultry bed bug populations that have been tested thus far. Therefore, it is advisable to test essential oil or OP products against small samples of 30–50 poultry house bed bugs to confirm susceptibility prior to the use of these insecticides in entire barns. Secondly, bed bugs tested in direct spray and residual bioassays as well as glass vial (Steelman et al. 2009, Ashbrook et al. 2017) and ceramic tile tests (Goddard and Maschek, 2015) were adequately exposed to insecticides as they had little or no chance to escape insecticide exposure by hiding or moving to untreated areas. However, as explained earlier, thorough treatment of all bed bug harborage areas in a large cage-free barn is difficult, which may afford bed bugs an opportunity to escape treated areas and survive insecticide treatments. Due to this latter factor, complete (100%) control of bed bugs infesting a poultry house using an insecticide-only approach may not be possible. While research on non-chemical control of bed bugs in poultry houses is lagging some techniques used are exclusion, monitoring and barn cleaning in-between poultry cycles (Axtell 1985, Mullens and Murillo 2017). The combined use of chemical and cultural control measures is likely more promising in achieving elimination of bed bug infestations from poultry barns.

2.5 Figures and Tables

Table 2.1. Probit estimated median lethal time (LT_{50}) values for different insecticides that were tested in direct spray bioassays against two bed bug populations (Harlan and PH2019).

Insecticide	**Strain**	**N**	**Chi-squared**	**df**	**P-value**	**Slope (±SE)**	**HF**	**LT_{50}[I] Days (FL95%)**[II] values in days	**LT_{50}[I] Resistance Ratio[III] at LT_{50} (95% CIs[III])**
EcoRaider (ready to use)	Harlan	200	15.34	5	0.0090	0.64 (0.105)	3.07	0.003 (0.00019– 0.012)	
	PH2019	200	10.11	5	0.0721	0.85 (0.089)	2.02	0.034 (0.013–0.07)	10.97* (3.19–37.72)
Tempo SC (0.05%)	Harlan	200	3.03	5	6.95	19.5 (181326)		0.41 (ND)[IV]	
	PH2019	200	8.95	5	0.1112	1.25 (0.09)		0.05 (0.035– 0.064)	0.12 (ND[IV])
Rabon (1.0%)	Harlan	200	18.35	5	0.0025	2.145 (0.28)	3.67	0.30 (0.18–0.49)	
	PH2019	200	6.89	5	0.229	1.92 (1.35)		35.9 (ND)[IV]	119.70* (3.05–4690.72)
Talstar (0.06%)	Harlan	200	0.64	5	0.99	2.24 (0.31)		0.05 (0.04–0.06)	
	PH2019	200	42.51	5	<0.0001	2.37 (0.52)	8.5	0.76 (0.23–1.48)	14.59* (5.66–37.64)

[I] LT_{50} = median lethal time necessary to kill 50% of individuals in days.
[II] FL = 95% fiducial limits.
[III] For resistance ratio calculations, the Harlan strain was used as the baseline population. Resistance ratios and their 95% confidence intervals (CIs) were calculated by using the method described by Robertson et al. 2017
[IV] The acronym ND indicates "not determinable".
* Statistically significant resistance ratios are marked with an asterisk (*). Resistance ratios were considered significant if their 95% CIs did not overlap with the value one.

Table 2.2. Probit estimated median lethal time (LT_{50}) values for different insecticides that were tested in residual bioassays against two bed bug populations (Harlan and PH2019).

Insecticide	**Strain**	**N**	**Chi–squared**	**df**	**P-value**	**Slope (±SE)**	**HF**	**LT_{50}[I] (FL 95%)[II]** values in days	**LT_{50}[I] Resistance Ratio[III] at LT_{50} (95% CIs[III])**
EcoRaider (ready to use)	Harlan	200	8.44	5	0.13	1.06 (0.08)		0.06 (0.04–0.08)	
	PH2019	200	12.27	5	0.03	1.13 (0.12)	2.45	0.11 (0.06–0.18)	1.83 (ND[IV])
Tempo SC (0.05%)	Harlan	200	5.46	5	0.36	3.49 (0.39)		0.2 (0.19– 0.27)	
	PH2019	200	72.4	5	<0.0001	1.22 (0.29)	14.48	0.13 (0.02–0.45)	0.57 (0.19–1.76)
Rabon (1.0%)	Harlan	200	31.1	5	<0.0001	2.54 (0.47)	6.22	0.8 (0.34– 1.32)	
	PH2019	200	4.7	5	0.45	0.78 (0.31)		686 (66.13– 4.8E10)	878.78* (20.31–38018.68)
Talstar (0.06%)	Harlan	200	136.87	5	<0.0001	2.95 (1.24)	27.37	0.12 (ND)[IV]	
	PH2019	200	73.78	5	<0.0001	2.41 (0.65)	14.76	0.66 (0.11– 1.66)	5.45 (0.87–34.02)

[I] LT_{50} = median lethal time necessary to kill 50% of individuals in days.
[II] FL = 95% fiducial limits.
[III] For resistance ratio calculations, the Harlan strain was used as the baseline population. Resistance ratios and their 95% confidence intervals (CIs) were calculated by using the method described by Robertson et al. 2017
[IV] The acronym ND indicates "not determinable".
* Statistically significant resistance ratios are marked with an asterisk (*). Resistance ratios were considered significant if their 95% CIs did not overlap with the value one.

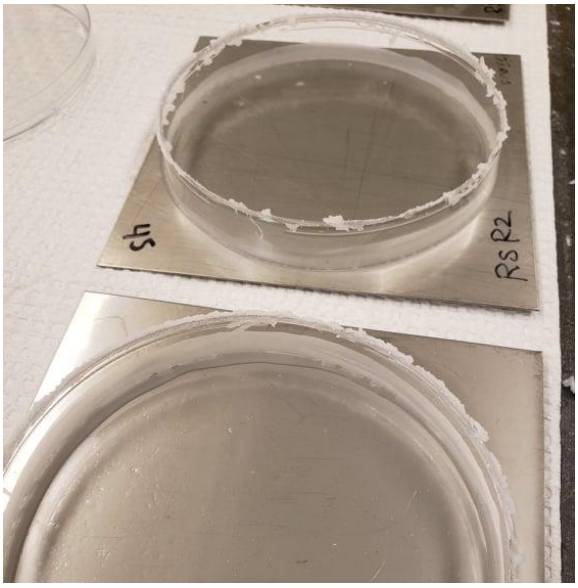

Figure 2.1. Shows the cut petri dishes glued onto the stainless-steel tiles to contain the bed bugs.

Figure 2.2. Shows the 60 ml spray bottle that was used to deliver insecticides or water in direct spray and residual bioassays.

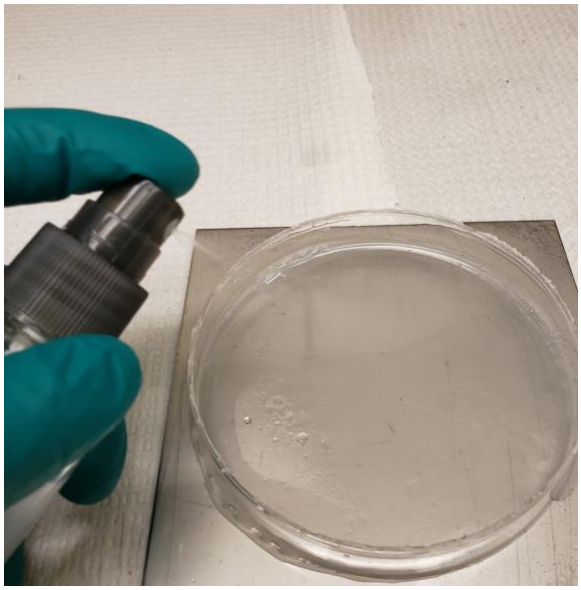

Figure 2.3. Shows the application method for the direct spray without bed bugs present.

Figure 2.4. Shows Whatman^{TM} #1 filter paper lining a 6 x 1.5 cm Petri dish.

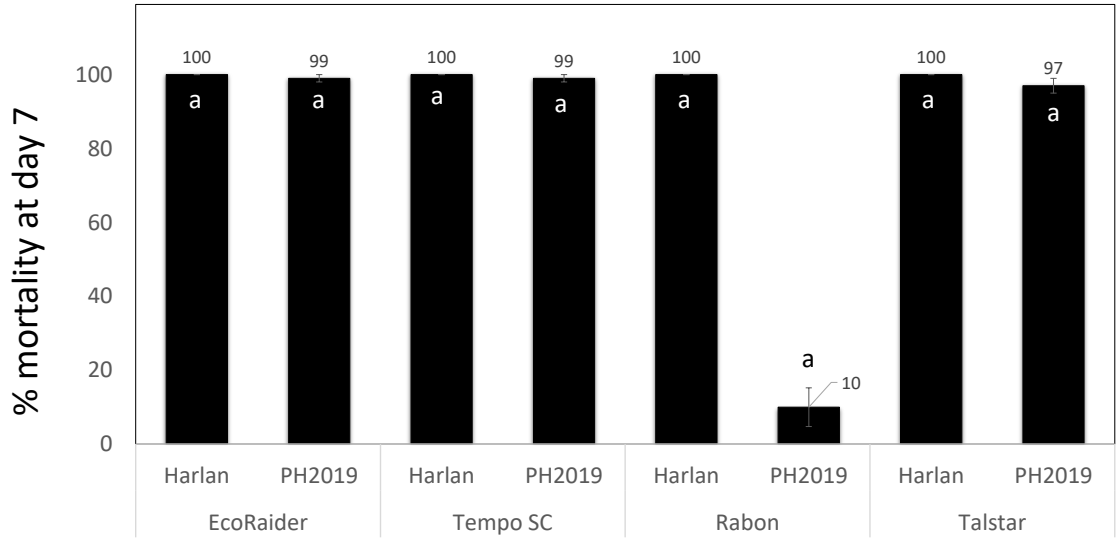

Figure 2.5. Depicts bed bug mortality for each product (EcoRaider, Tempo SC, Rabon, Talstar) on the 7th day of the direct spray bioassay. For each insecticide, bars connected with same letters indicate lack of statistical differences in mortality between Harlan and PH2019 populations (P<0.05; student's t-test).

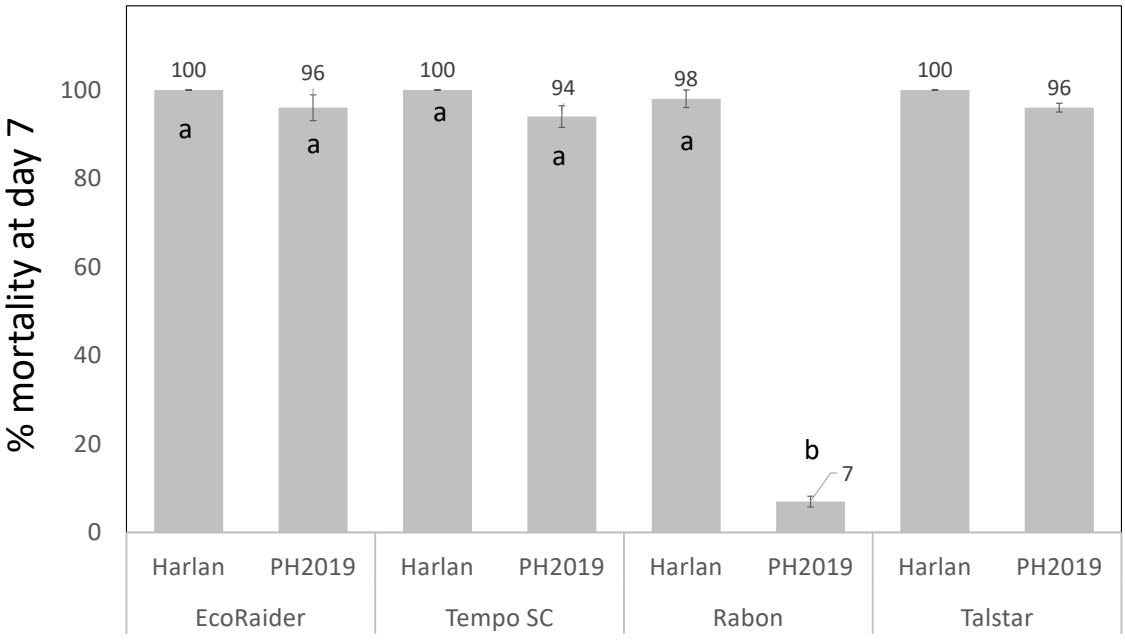

Figure 2.6. Depicts bed bug mortality for each product (EcoRaider, Tempo SC, Rabon, Talstar) on the 7th day of the residual bioassay. For each insecticide, bars connected with same letters indicate lack of statistical differences in mortality between Harlan and PH2019 populations (P<0.05; student's t-test).

CHAPTER 3. CONTAMINATION OF STAINLESS-STEEL TILES WITH POULTRY BARN DEBRIS DOES NOT AFFECT INSECTICIDE EFFICACY AGAINST BED BUGS

3.1 Introduction:

Conventional cages are not viable environments for ectoparasites because there is a lack of cage to soil contact and large cracks to harbor pests such as bed bugs (Mullens & Murillo, 2017). Cage-free poultry systems are vulnerable to bed bugs by having many areas where the bed bugs can harbor and reproduce. Common hiding areas include alongside perches, nest sites where poultry congregate, rails of slat platforms, inside nesting boxes, under nest pads, and between slats of the support beams (Machtinger & Martin, 2021). Additionally, bed bugs can hide in any cracks and crevices within the walls and burrow into ceiling insulation (Machtinger & Martin, 2021). Bed bug infestations in cage-free housing systems are becoming more frequent and the control of the infestations becomes more plausible than complete eradication in most cases (Maschek, 2015). There are many cracks and crevices for bed bugs to hide within the poultry systems and other application difficulties such as treating within an environment full of debris such as dust, chicken manure, unstable moisture levels and where floors of the barn are built on soil (Mullens and Murillo, 2017). The relationship between monitoring and control becoming increasingly important when treating bed bug populations (Mullens and Murillo, 2017). To assess some of these concerns in addition to answering initial questions in objective one, the goal of the second objective was to test product efficacy on a substrate contaminated with debris collected from a poultry barn. This is the first study that compares effect of debris on efficacy of insecticides products used for control of bed bugs in poultry barns.

3.2 Materials & Methods

3.2.1 Bed bug Strains

All bioassay experiments to compare clean versus contaminated substrate were conducted using the field collected PH2019 strain. The PH2019 strain was collected from a cage free layer hen facility in the Midwest region in June 2019. Since its collection from the field in 2019, it has been lab-adapted and has been reared in the laboratory at Purdue University using defibrinated rabbit blood (Hemostat Laboratories, Inc., Dixon, CA) as a blood source. The population was maintained at 27 1°C, 50 ± 10% RH and a 12:12h (L:D) cycle in a temperature controlled environmental chamber (Percival Scientific, Perry, IA). PH2019 bed bugs were fed on defibrinated rabbit blood purchased from Hemostat Laboratories in Dixon, CA. The parafilm membrane feeding method (Chin-Heady et al. 2013) was used to feed the bed bugs.

3.2.2 Chemicals

As in objective one, the insecticide products used in objective two included Talstar® Professional, Tempo® SC and Rabon® and chemicals were used at their label-recommended dilution rates.

3.2.3 Contamination of Tiles with Chicken Manure

Chicken manure was collected from a small poultry shed housing approximately 10 chickens in the Midwest region. The poultry shed was never infested with bed bugs, but the chickens had received a Piperazine water treatment for nematodes three months prior to manure collection. The manure was dried (100ºC) and ground before use in bioassays. About 0.1 g of manure was applied to each stainless-steel tile (4 x 4 inches in dimensions) affixed with a plastic Petri dish base. The manure on the tile was then moistened with 1 mL of distilled water using a 60

mL spray bottle (Meijer). The bottle was held at a distance of 5–10 cm from the substrate. The manure-contaminated tiles were allowed to dry for at least 30 minutes or until they were dry.

3.2.4 Direct Spray Bioassays

Stainless steel tiles contaminated with manure as well as clean tiles were used for direct spray bioassays. The contaminated steel tiles mimicked the substrate the bed bugs would encounter in a cage-free barn. Each bioassay replicate representing a single stainless-steel tile included 20 bed bug adults (males and females in 1:1 ratio). Bed bugs were confined on the stainless-steel tile by using an open-ended base or bottom part of a 100 x 15 mm plastic Petri dish (Falcon, Hainesport, NJ) as shown in Fig. 2.1. The open-ended Petri dish base was affixed to the tile using glue (Elmer's, Westerville, OH). Insecticide application volume was equivalent to the standard rate of 1 gallon per 1000 square feet, which translated to 0.42 mL of water-diluted or ready to use insecticide or essential oil product for each 4 x 4-inch tile. This amount was administered using a 60 mL spray bottle (Meijer, Grand Rapids, MI) (Fig. 2.2).

After spray treatment, bed bugs were held on the steel tiles for five minutes. After 5 minutes had elapsed, they were transferred to a filter paper (Whatman #1.55 mm) lined 60 x 15 mm Petri dish (Falcon) (Fig. 2.4). Control bed bugs were sprayed with 0.42 mL of distilled water and were subsequently processed using procedures described above for different insecticides. As explained above, each pesticide and control treatment had a clean and contaminated tile for efficacy comparison. Each treatment with different insecticides and contaminated or clean tiles was replicated 4 times. Post direct spray treatment, Petri dishes containing bed bugs were held in temperature controlled environmental chambers that were also used for rearing bed bugs. Mortality was recorded at 5 minutes, 30 minutes, 4 hours, 1 day, 3 days, 5 days, and 7 days. Bed bugs showing immobility when prodded with a pair of forceps.

3.2.5 Residual Bioassays

Bioassays with dried residues of three synthetic insecticides and one essential oil product were also conducted with manure-contaminated and clean stainless-steel tiles (Fig. 3.1). The same chemical dilution procedure as described under objective 1 was followed. A 60 mL hand sprayer (Meijer) was used to treat tiles with 0.42 mL of water-diluted or ready to use pesticides. Insecticide blank or control tiles were treated with 0.42 mL of tap water. After treatment, chemical and water-treated tiles were allowed to dry overnight (16–24 h) at room temperature (21–22°C). After the tiles were dry, 20 adult bed bugs (1:1 ratio of males: females) were released and maintained on the tiles for 4 hours. The open plastic Petri dish ring prevented bed bugs from escaping the insecticide or water-treated area of the tile . After the 4-hour exposure on treated or control tiles, bed bugs were transferred to Whatman filter paper lined 60 x 15 mm plastic Petri dishes (Falcon). Four replicates with 20 adult bed bugs each were conducted for each insecticide and control or water treatments as well as for clean and contaminated tiles. Petri dishes were then placed in an environmental chamber that was used for rearing. Mortality recording started while the bed bugs were confined on the tiles (30 minutes, 1 hour and 4 hours). Subsequent mortality observations were recorded at 1 day, 3 days, 5 days, and 7 days.

3.2.6 Data Analysis

The data on survivability of PH2019 in the clean versus unclean treatments was used to generate Kaplan-Meier survivability curves and the statistical significance of these curves was compared using log rank test in JMP PRO 15.1. Prior to conducting survivability analysis, the survivorship data was adjusted using Abbott's Formula to account for control mortality.

3.3 Results

3.3.1 Survivorship of the PH2019 Strain Bed Bugs on Contaminated Vs. Clean Tiles in a Direct Spray Bioassay

The direct spray bioassays resulted in control mortality of 13.75% mortality in clean and contaminated tiles at 7 days. These insects died naturally without exposure to any insecticide but were sprayed with distilled water. Kaplan-Meier survivability curves for Talstar, Tempo SC, EcoRaider, and Rabon were generated using the PH2019 strain with the formula in JMP Pro 15.1 (Figure 3.2). Talstar exhibited a slight difference between contaminated tiles and clean tiles (Fig. 3.2.1). Tempo SC and EcoRaider did not present any difference in survivability among the two tile types (Figs. 3.2.2 and 3.2.3) . Rabon showed the highest level of percentage survivorship difference between a contaminated tile and the clean tile (Figure 3.2.4). None of the four p-values (Talstar = 0.84, Tempo SC = 1.00, EcoRaider = 1.00, Rabon = 0.05) showed statistically significance. However, as it can be seen from the p-value for Rabon, the survivorship differences between clean and contaminated tiles were close to being statistically significant (p-value of 0.05).

3.3.2 Comparative Survivability of the PH2019 Strain on Contaminated Vs. Clean Tiles Residual Bioassays

Average control mortality of 12.5% was observed for clean tiles and it was 11.25% for contaminated tiles during the 7-day residual bioassay experiment. Just as in the direct spray bioassays, survivability graphs were generated using the Kaplan-Meier formula in JMP Pro 15.1. As shown in Figure 3.3. Talstar and Tempo SC did not show any differences between survivability on a clean tile versus a contaminated tile (Figs. 3.3.1 and 3.3.2). In the residual bioassay, both EcoRaider (Figure 3.3.3) and Rabon (Figure 3.3.4) differed numerically in survivorship percentages across the 7-day bioassay period. However, none of the -values were statistically

significant (Talstar = 1.00, Tempo SC = 1.00, EcoRaider = 0.1637, Rabon = 0.064). However, Rabon showed the closest p-value to statistical significance and possibly suggests there may be a difference between the two tile types. Further research on this topic would be needed to verify if Rabon has lower efficacy on contaminated or porous substrates.

3.4 Discussion

Substrate is a key factor that can impact product efficacy (Chadwick 1985, Wang et al. 2016). A non-porous substrate is a surface that is smooth with the inability of water absorption. A stainless-steel tile is considered a non-porous surface. Soil and manure are considered as porous as they can retain and absorb water. The tiles used in this study were the combination of a non-porous substrate with a thin, porous layer of debris on the top. In general, a more porous substrate reduces product efficacy in comparison to a less porous substrate. Wang et al (2016) conducted residual efficacy experiments with bed bugs on four substrates: fabric, unpainted birch plywood, painted birch plywood, and vinyl. They forced the bed bugs to be exposed to the insecticides for four hours (Wang et al. 2016), which is similar to exposure time used in this study. The four products tested by Wang et al. were Tandem, Temprid SC, Transport GHP, and Demand CS (Wang et al. 2016). Our study focused on mimicking field settings, where the poultry barns constructed with stainless steel are routinely contaminated with dirt, manure, and other debris (Mullens and Murillo, 2017). In Wang et al (2016) there was no consistent pattern between product efficacy and the substrate porosity except for Tandem that overall performed more poorly on more porous substrates. Similar results were observed in our study, with all four insecticides not showing any differences in efficacy between the contaminated and clean substrates. Product efficacy for Talstar (Figure 3.3.1) and Tempo SC (Figure 3.3.2) did not change when administered on the two types of substrates in the residual. The p-values were non-significant (Talstar = 1.00, Tempo SC = 1.00).

EcoRaider (Figure 3.3.3) and Rabon (Figure 3.3.4) did show a higher percentage survival on the contaminated tiles in the residual. However, the p-values were not significant (Rabon = 0.064, EcoRaider = 0.1637). An explanation for why EcoRaider seemed to show slightly better product efficacy on the clean tiles was because during the 24-hour drying period some of the essential oil appeared to have been absorbed by the debris on the contaminated tile. This observation was not made during the direct spray bioassay. Although none of the p-values were significant in the log-rank test, the findings do indicate the possibility of statistical significance if repeated with higher amounts of debris. Especially for the insecticide Rabon which had a p-value closest to a significant value in the residual bioassay (Figure 3.3.4). If repeated, the amount of contaminants could be increased, as 0.1g per tile was likely not been enough to alter the effectiveness of the formulated insecticides. In conclusion, tile contamination did not significantly affect the efficacy of formulated insecticides, at least when the contamination level only forms a light or thin layer of dust on the test substrate, which is what is observed in poultry barns.

3.5 Figures and Tables

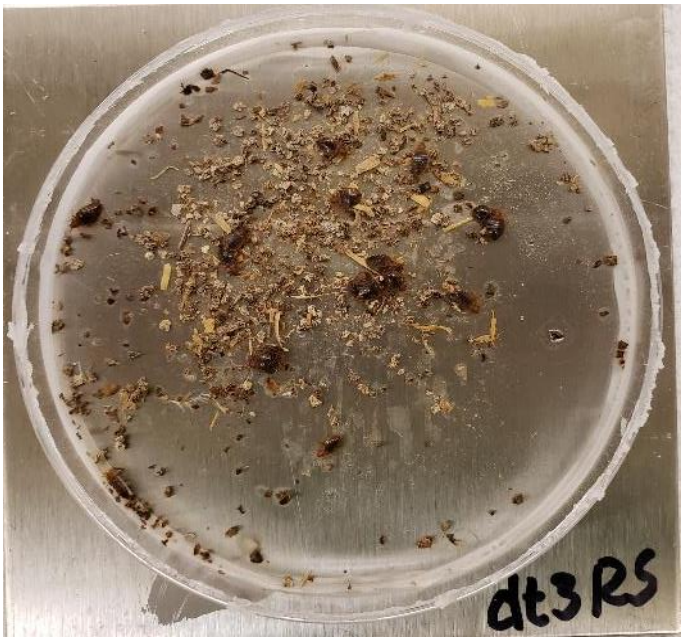

Figure 3.1. A petri dish contaminated with chicken manure that is being used for a residual treatment.

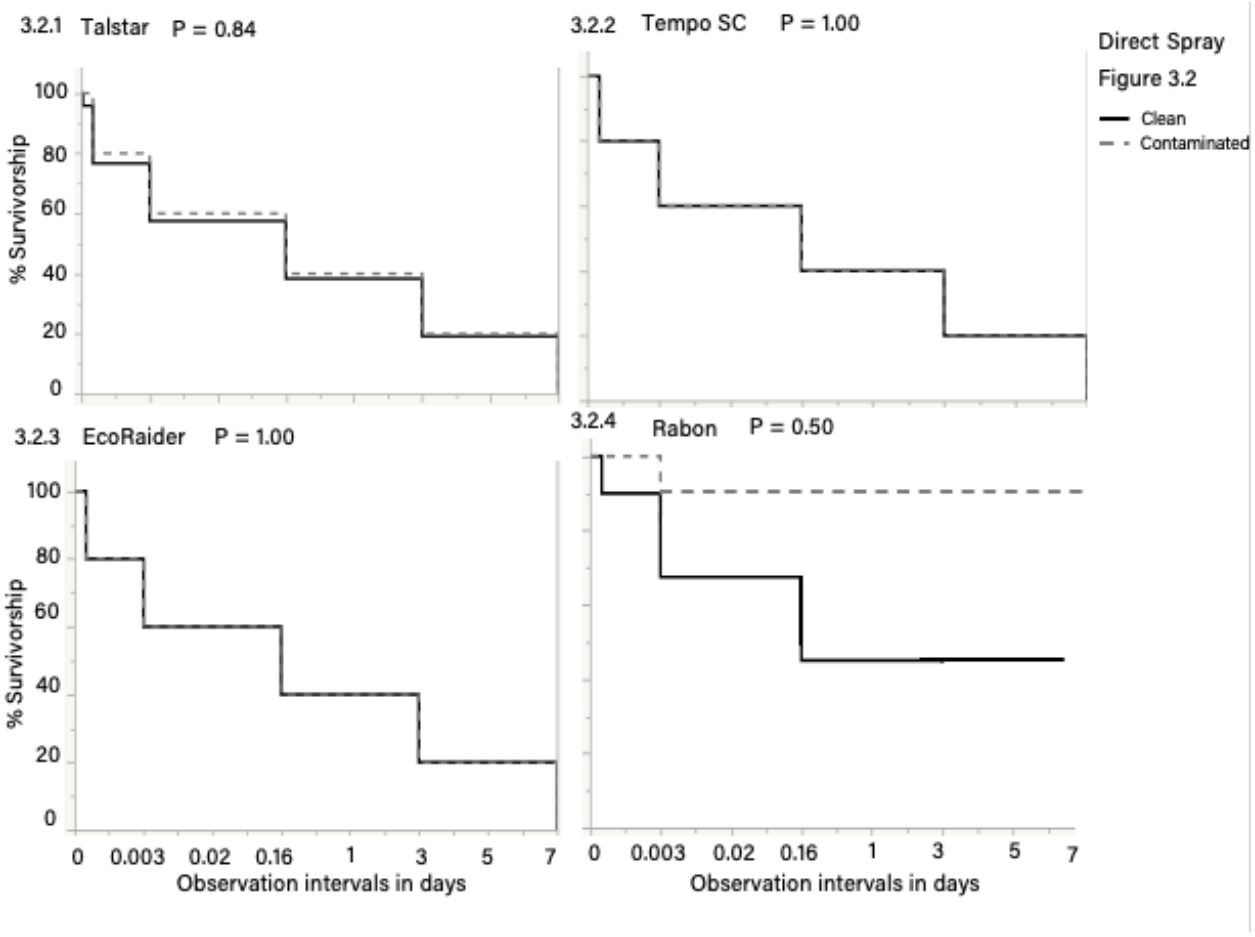

Figure 3.2. Kaplan-Meier survivorship curves for different insecticides tested against the PH2019 population in direct spray bioassays. The solid lines represent bed bug survivability when treatments were applied to a clean tile and the dashed line represents a treatment applied to a contaminated tile. Statistical comparison of survivorship of bed bugs on clean versus contaminated tiles was performed using the log-rank test at the significance level of $P<0.05$

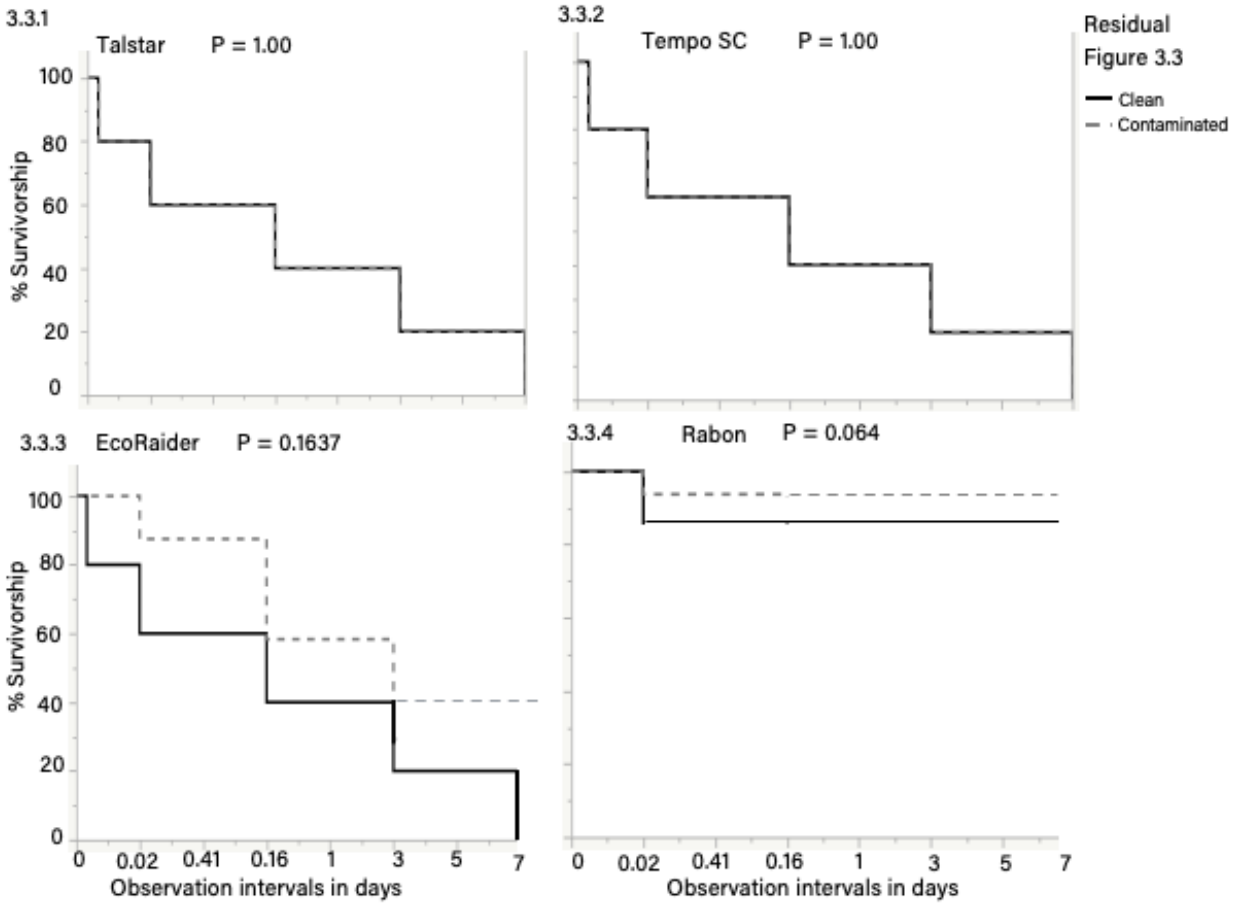

Figure 3.3. Kaplan-Meier survivorship curves for different insecticides tested against the PH2019 population in residual bioassays. The solid lines represent bed bug survivability when treatments were applied to a clean tile and the dashed line represents a treatment applied to a contaminated tile. Statistical comparison of survivorship of bed bugs on clean versus contaminated tiles was performed using the log-rank test at the significance level of $P<0.05$

CHAPTER 4. CONCLUSION

In the first phase of our experiment, I tested the efficacy of four insecticide products that are registered for use in poultry houses on a susceptible and field bed bug strain. Our studies indicated that the PH2019 strain may have been previously exposed to organophosphates. Because it consistently displayed a much slower response to Rabon in both direct spray and residual bioassays. Probit analysis suggested a LT_{50} of approximately 35.9 and 686 days for PH2019 for the direct spray and residual treatment of Rabon (Table 2.1, Table 2.2). In combination with the slow response to the chemical, the resistance ratios were large and statically significant at 119.70 (Table 2.1) and 878.78 (Table 2.2). There is no strong indication that pyrethroids (Talstar Professional and Tempo SC) would not be effective in controlling the PH2019 population which is similar to results from previous studies (Goddard and Maschek, 2015). The only downside for Tempo SC use is that it cannot be applied while poultry are present in the houses (Goddard and Maschek, 2015). EcoRaider performed well in the first phase of experiments, showing only a slightly slower impact during the residual bioassay, however other research on essential oils suggest that it may have a short residual efficacy (Gaire et al. 2019, 2021). Although EcoRaider may have a field efficacy of above 90% in human dwellings (Wang et al. 2014), complete bed bug elimination was not achieved in most cases suggesting that essential oils are not the most effective treatment inside poultry houses.

In the second phase of our experiments, I tested the survival of the PH2019 strain when the tiles were contaminated with poultry house chicken manure and debris to compare the differences between a laboratory and simulated field setting. Although none of the p-values were statistically significant there is additional indication that Rabon performs better on a clean substrate than a contaminated substrate. Survivorship was higher for the bed bugs treated on a contaminated tile.

Both pyrethroids performed similarly if not the same on the two-substrate types. EcoRaider did show a difference in survivorship in the residual bioassay; I believe this is linked to the properties of it being an oil. The product was seemingly absorbed by pieces of clumped debris, which was easily avoided by the bed bugs. An essential oil product may not be the best treatment for the field even though it performed well in laboratory trials. In conclusion, the best treatment plan for cage-free poultry houses is likely a pyrethroid insecticide such as Talstar Professional that can be administered while birds are present and Tempo SC Ultra that can be used when chickens are not present. Nonetheless, there is also a need to perform additional research on non-chemical or alternative control measures for the control of bed bugs infesting poultry houses because sole reliance on the use of insecticides for bed bug control lead to insecticide resistance issues and also environmental contamination or nontarget effects.

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