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Insecticide Resistance in the Bed Bug

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INSECTICIDE RESISTANCE IN THE BED BUG

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DISSERTATION

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A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in the College of Agriculture, Food and Environment at the
University of Kentucky
By

Jennifer R. Gordon

Lexington, Kentucky

Director: Dr. Kenneth F Haynes, Professor of Entomology

Lexington, Kentucky

ABSTRACT OF DISSERTATION

INSECTICIDE RESISTANCE IN THE BED BUG

Populations of *Cimex lectularius*, the bed bug, have resurged around the world posing significant challenges for pest management professionals and causing physical, economic, and emotional strife. Pyrethroid resistance has been found in the vast majority of populations making pest management more difficult. The objectives of my dissertation research were to document the evolution of resistance to pyrethroid and neonicotinoid combination products (called combination products here) and to a neonicotinoid in the laboratory, to record potential fitness costs to resistance to the combination products, and to compare the efficacy of nine insecticides on six populations. In the laboratory, populations of bed bugs evolve resistance rapidly to a combination product and that resistance translates into cross resistance to another combination product. In a follow up experiment, resistance to a neonicotinoid occurred after three generations of selection. Cross resistance between neonicotinoid and pyrethroid resistance was also found, likely due to a common detoxification mechanism (cytochrome P450 mediated metabolism). Resistance was associated with life history costs in three populations that had been selected with a combination product. Therefore, in the absence of selection pressure, populations of bed bugs should revert towards increasing susceptibility. Two pyrethroid products and three combination products were effective at killing three populations of bed bugs but were relatively ineffective against three other populations. However, the combination product, Transport GHP®, the single action pyrrole product, Phantom SC®, and the single action desiccant, CimeXa®, killed 95 to 100% of all populations investigated over a 14-day exposure. Taken together, results reported in this dissertation suggest that insecticide resistance management may be a useful tool for extending the efficacy of insecticides for control of *C. lectularius*.

INSECTICIDE RESISTANCE IN THE BED BUG

By

Jennifer R. Gordon

2014

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__8 December 2014_____
Date

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Chapter 1. Introduction

Bed bug biology

Populations of the bed bug, *Cimex lectularius*, have reemerged globally during the 21st century after a nearly 50 year period of near absence in Canada, North America, Europe and Australia (Eddy and Jones 2011). Before the decline in populations, much was known about this bug (Usinger 1966). Bed bugs are dorso-ventrally flattened, brownish-red insects that belong to the order Hemiptera and the family Cimicidae (Usinger 1966). The bugs are hemimetabolous consisting of three stages (egg, nymph and adult) and five instars (Usinger 1966). Longevity can be greater than 12 months, and bed bug females can have high reproductive rates (Usinger 1966). Each instar requires a blood meal to further development, and adult bed bugs require blood meals for oogenesis and spermatogenesis (Usinger 1966, Reinhardt and Siva-Jothy 2007) making this bug an obligate parasite. Ramifications of bed bug life history and infestations can be severe and directly impact people by causing physical and emotional distress, economic hardships and negative societal consequences.

Impact of bed bugs

The potential for disease transmission and the physical and emotional distress associated with living with populations of bed bugs cause this bug to be of significant importance to public health. Bed bugs are not known to actively vector disease; however, passive transmission of mutli-drug resistant *Staphylococcus aureus* (MRSA) and *Trypanasoma cruzi*, the causative agent of Chagas disease, have been implicated in the laboratory (Shaw 2011; Salazar et al. 2014). Human reactions to bed bugs bites vary ranging from no reaction to severe localized reactions and even death (Potter et al. 2010a); however, death due to anaphylaxis is rare (Goddard and deShazo 2009). Often,

the psychological problems attributed to living with an infestation of bed bugs can be just as serious as the threat to physical health. One survey asked individuals with confirmed bed bug infestations what symptoms they attributed to these populations other than allergic responses to the bites, and the respondents cited insomnia and various other stresses (Potter et al. 2010). Additionally, the emotional ramifications can be more severe and include delusional parasitosis and thoughts of suicide (Goddard and deShazo 2009).

Infestations of bed bugs can have severe economic and social impacts in the form of extermination costs, decreases in productivity, damage to brand names and lawsuits. The average cost to treat a bed bug infestation in a single family residence is $\geq$ $500 (Potter et al. 2010) and to heat treat an apartment costs between $800-1200 (Stedfast and Miller 2014). Taken as a whole, millions of dollars per year are spent trying to control this pest in private residences (Potter and Haynes 2014). Similarly, the hospitality and agricultural industries spend millions of dollars on control due to the financial impact this pest has on the damage to brand names and product (Axtell 1999, Reinhardt and Siva-Jothy 2007, Doggett et al. 2012). In addition to costs incurred by the presence and control of this pest, millions of dollars are spent on bed bug litigation (Doggett et al. 2012). Finally, people with bed bugs in their homes often face social rejection and damaged reputations. Given the adverse social, economic and health related issues associated with this pest, many tactics have been developed to control this pest.

Classes of insecticides

Chemical insecticides are the most common method used to control populations of *C. lectularius* (Potter et al. 2011). In the past, wide spread use of DDT and other

chlorinated insecticides followed by the rotation to other highly effective classes (organophosphate, carbamate and pyrethroid) probably caused the decline in infestations (Potter 2011). Many of these classes are no longer registered for use in most countries. Chlorinated hydrocarbon insecticides (CHC) contain at least one chlorine attached to a carbon and generally affect the central nervous system (O'Brien 1970). These insecticides are known for their fast action against insects, high toxicity and long residual activity. Some CHCs share a common target site with pyrethroids (sodium ion channels; O'Brien 1970), and selection for target site mediated resistance by one compound should result in cross resistance to the other. Two of the other groups of insecticides used against bed bugs in the past (organophosphates and carbamates) inhibit acetylcholine esterase (AChE), an enzyme always present in the synapse that metabolizes the neurotransmitter acetylcholine, resulting in paralysis and death (O'Brien 1970). High mammalian toxicity and long environmental persistence has led to the ban or limited use of most organophosphates, carbamates and CHCs (Costa 2006, Fishel 2014).

Today, multiple classes of insecticides are registered for bed bug control globally (Wang and Wen 2011, Doggett 2013, Potter and Haynes 2014). Currently, insecticidal formulations containing a pyrethroid are the most commonly used products against this pest (Wang and Wen 2011, Potter et al. 2012, Doggett 2013, Potter and Haynes 2014); however, other classes including the neonicotinoids, pyrroles, desiccant dusts, insect growth regulators, organophosphates, and carbamates are also used worldwide (Wang and Wen 2009, Romero et al. 2010, Doggett 2013, Goodman et al. 2013, Gordon et al. 2014a, Potter et al. 2014). Both pyrethroids and neonicotinoids are neurotoxins acting at different target sites on the neuron. Pyrethroids kill by interfering with sodium ion

channels along the nerve axon (Soderlund and Bloomquist 1989); whereas neonicotinoids bind to nicotinic acetyl choline receptors on the postsynaptic membrane (Tomizawa and Cassida 2005). Unlike pyrethroids, the use of neonicotinoids is restricted and limited to fewer countries (Wang and Wen 2009, Doggett 2013, Potter and Haynes 2014). Combination products utilizing both of these active ingredients are favored in the field (Potter et al. 2011), but the evolution of insecticide resistance in the field is a concern (Gordon et al. 2014a).

Some other less commonly used insecticides include the pyrroles, desiccant dusts, insect growth regulators, carbamates and organophosphates. The pyrroles act by interrupting the electron transport chain in the mitochondria of all cells (Romero et al. 2010). Research contained within this dissertation found that one product containing the pyrrole chlorfenapyr ultimately controlled 100% of populations challenged in the laboratory. Desiccant dusts act by adhering to the insect cuticle and adsorbing lipids, which accelerates desiccation (Doggett et al. 2012). Two examples of desiccant dusts are diatomaceous earth and amorphous silica gel; however, research from the field indicates that silica gel is the more effective product (Potter et al. 2014). Insect growth regulators mimic insect hormones and disrupt normal biological functions by preventing eclosion and molting, however, the overall effectiveness of these products is debated (Moore and Miller 2009, Goodman et al. 2013). Regardless of country, chemical control methods in general are favored by pest management technicians due to the ease of use and residual properties of many insecticides. However, nonchemical control methods can sometimes be equally or more effective and are not regulated by government agencies.

Alternative control tactics

Management of *C. lectularius* often requires many methods including early detection, mechanical removal and the use of heat and steam. Early detection of small, localized infestations of bed bugs can be instrumental at effectively eliminating this pest, because locating and managing all members of a small population is easier compared to a large infestation. To detect bed bugs, pest management professionals (PMPs) use traps and trained dogs. Intercepting traps (often resembling pitfall traps) capture bed bugs as they move to and from the bed or around the floor. They allow PMPs to detect bed bug presence and sometimes give information about the source location (Doggett et al. 2012, Wang et al. 2009a). In addition to passive traps, active traps use chemical attractants, carbon dioxide and/or heat (Wang et al. 2009b). Dogs trained to react to the smell of bed bugs have also been used to detect their presence (Pfiester et al. 2008). Upon the discovery of an infestation, a significant proportion of the infestation can often be removed through mechanical means. Homeowners can eliminate a portion of the population by entombing bed bugs with mattress encasements and creating a permanent barrier between the bugs and the person (Koganemaru and Miller 2013). Similarly, vacuuming has been proven effective at removing bed bugs. Unfortunately, people living with bed bugs can only mechanically remove bugs that can be found; however, eliminating an infestations requires the control of all members of a population, especially hidden individuals. Heat and steam have been demonstrated to control populations and can frequently kill bugs that were not detected during the initial inspection (Potter et al. 2008, Pereira et al. 2009, Potter et al. 2011 Doggett et al. 2012).

Insecticide resistance

Insecticide resistance is a world-wide phenomenon that has been observed in hundreds of insect species of medical, urban and agricultural importance (Melander 1912, Forgash 1984, Hemmingway and Ranson 2000). Any chemical that kills applies selection pressure, which together with genetic heterogeneity within the population, results in evolution that may translate into the loss of effectiveness of the insecticide. In a naïve population, susceptibility is normally distributed with a small proportion of individuals dying at low concentrations and an equally low proportion of individuals surviving exposure to high concentrations of the same insecticide. Individuals that survive an insecticide treatment are the progenitors for the next, more resistant generation. Increasing levels of resistance with repeated applications reflect an increasing proportion of the population containing alleles conferring resistance. This "treadmill" may continue until the insecticide fails to control the population, sometimes with several molecular mechanisms of resistance contributing to that result (Mamidala et al. 2011, Zhu et al. 2013).

Molecular mechanisms of insecticide resistance fall within three categories: reduced cuticular penetration, target site insensitivity and enhanced metabolism (Hemmingway and Ranson 2000, Mamidala et al. 2011). Changes to the insect cuticle can decrease the rate of penetration into the hemocoel allowing the pest more time to detoxify or eliminate the insecticide. One study investigated penetration of the pyrethroid deltamethrin into *Helicoverpa armigera*, the cotton boll worm, and found that the time required for a susceptible cuticle to absorb 50 % of the applied insecticide was 1 h but the cuticle of a resistant strain took 6 h (Ahmad et al. 2006). Similarly, Wood et al.

(2010) found that there was a positive correlation between cuticle thickness and the time for knockdown in resistant *Anopheles funestus*. Whereas reduced cuticular penetration results in significant loss of susceptibility, more frequently, maximal resistance is associated with (co)expression of reduced target site sensitivity and/or enhanced metabolism.

Mutations of genes resulting in the production of altered target sites can convey a level of resistance if the new phenotype results in the insecticide's inability to interact lethally. As the target site of pyrethroids and DDT, point mutations that alter sensitivity of voltage gated sodium channels have been extensively studied and much is known about the mutations that cause knockdown resistance and super-knockdown resistance (kdr and super-kdr). The phenomenon of kdr refers to a single point mutation and usually results when a switch from leucine to one of three different amino acids (phenylalanine, histidine or serine) occurs at the S6 hydrophobic segment of domain II of insect sodium ion channels (Williamson et. al. 1996, Park and Taylor 1997, Martinez-Torres et. al. 1999); however, other novel point mutations can occur and result in a similar resistance (Yoon et al. 2008). Super-kdr refers to the addition of a second point mutation in conjunction with the leucine switch (Guerrero et al. 1997). Sodium ion channels are not the only target site that loses sensitivity due to point mutations. A single point mutation of AChE can convey a high degree of resistance to both organophosphates and carbamates (Weill et. al 2004). Fixation of target site mutations within a population can lead to the loss of entire classes of insecticides forcing stakeholders to utilize alternative classes. However, selection favoring metabolic resistance can lead to the loss of multiple classes of insecticides.

Enhanced metabolism results from altered expression of one of three major classes of detoxifying enzymes: glutathione S-transferases (GST), P450 monooxygenases and esterases. A change in enzymatic activity may be either qualitative or quantitative. A qualitative change in an enzyme results in expression of a mutant, detoxifying enzyme with a heightened affinity for an insecticide. For example, a mutant carboxylesterase in *Culex tarsalis* exhibited an increased ability to hydrolyze malathion (Ziegler et al. 1987). Similarly, a mutant GST could dechlorinate DDT in a resistant strain of the house fly, *Musca domestica* (Clark and Shamaan 1984). Whereas qualitative changes in enzymes convey resistance, more commonly, quantitative changes in enzyme expression occurs. Overproduction of a wild-type, insecticide-detoxifying enzyme by either gene amplification or upregulation occurs for all three classes of detoxifying enzymes (Hemingway et al. 1998). The enzymes often have slow catalytic rates but act as a sink and prevent the insecticide from interacting with the intended target site. Devonshire and Moores (1982) showed that 3 % of the total proteins of *Myzus persicae*, the peach aphid, were esterases that conveyed cross-resistance to organophosphates, carbamates and pyrethroids. The mechanism of enzymatic overproduction occurs through either gene amplification or modification of transcription factors (Devonshire and Moores 1982, Zhu et al. 2012). In resistant *Culex quinquefasciatus*, Mouches et al. (1986) originally estimated that there were 250 copies of an esterase gene. More commonly, however, over-expression of detoxifying enzymes occurs via upregulation of a gene coding for an enzyme through an alteration in the transcription of the gene (Hemingway and Ranson 2000, Zhu et al. 2012). Since the resurgence in populations of insecticide resistant bed bugs, much effort has been spent investigating the underlying molecular mechanisms.

Insecticide resistance in *C. lectularius*

Insecticide resistance in the bed bug involves multiple molecular mechanisms of resistance. Changes in the bed bug cuticle have been implicated in conveying a level of resistance in populations of bed bugs by delaying absorption and producing metabolizing enzymes in epidermal cells (Koganemaru et al. 2013, Zhu et al. 2013). One study found genes associated with the bed bug cuticle were upregulated in resistant strains compared to susceptible, and that susceptibility to a pyrethroid was significantly increased when the mode of administration changed from topical application to injection (Koganemaru et al. 2013). Another study confirmed the upregulation of cuticular genes but also found that the epidermal cells of the cuticle were producing enzymes involved in actively metabolizing insecticides (Zhu et al. 2013). The production of metabolizing enzymes via the cuticle is unique to the bed bug and has not been documented in any other insect. Similarly, decreased target site sensitivity has been recorded in resistant populations of *C. lectularius*. Zhu et al. (2010) surveyed populations of bed bugs from across the United States for *kdr* mutations and found that 89% of all populations had at least one mutation. Insecticide detoxification has been implicated in many resistant populations of *C. lectularius* (Yoon et al. 2008, Romero et al. 2009, Adelman et al. 2011, Mamidala et al. 2011, Zhu et al. 2012, 2013). Of the three classes of enzymes generally involved in insecticide resistance, the esterase and cytochrome P450 classes are the most important. Recent studies have implicated at least two different esterases involved in detoxification of pyrethroids (Adelman et al. 2011, Zhu et al. 2013); however, esterase involvement is still unclear as one study could find no implication of this class' involvement in insecticide resistance (Yoon et al. 2008). On the contrary, the majority of studies

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investigating the molecular mechanisms of pyrethroid resistance in populations of *C. lectularius* have obtained evidence to implicate P450s (Yoon et al. 2008, Romero et al. 2009, Adelman et al. 2011, Bai et al. 2011, Zhu et al. 2012, 2013).

Fitness costs due to insecticide resistance

Fitness costs due to insecticide resistance are manifested in physiological and behavioral traits. Physiological fitness costs of insecticide resistance may be reflected in traits such as decreases in longevity, fecundity, body mass, and/or increases in development time (Carriere et al. 1994, Mebrahtu et al. 1997, Liu and Han 2006, Pereira et al. 2011, Martins et al. 2012, Kliot and Ghanim 2012, Otali et al. 2014). Resistant populations of *Aedes aegypti* collected from Brazil and selected in the laboratory all experienced a decrease in female longevity and reduction in eggs laid (Martins et al. 2012). Behavioral costs have also been observed with insecticide resistance (Kliot and Ghanim 2012). One study investigating *M. persicae* found that resistant aphids were less likely to leave senescing leaves and less able to respond to alarm pheromone compared to susceptible aphids, which could favor susceptible alleles in environments not containing insecticides if resistant aphids died from starvation or predation (Foster et al. 2003). The same study found that insecticide resistant house flies, *M. domestica*, were less able to respond to temperature gradients compared to susceptible, which could increase the risk of mortality to resistant individuals by being trapped in cold environments (Foster et al. 2003). Quantifying the tradeoffs between life history and insecticide resistance is only part of the story. To understand the evolution of insecticide resistance, one must also understand the mechanisms of fitness costs as well (see Chapter 6).

Mechanisms of fitness costs

Tradeoffs between life history and insecticide resistance can be driven by either an accumulation of antagonistically pleiotropic genes and/or a linkage disequilibrium between genes affecting the level of resistance and other fitness parameters. Sometimes these genetic mechanisms manifest as a tradeoff between a finite amount of resources such as energy spent on body size verses longevity (Stearns 1989, Roff 2002). Antagonistic pleiotropy occurs when the same gene that influences insecticide resistance also negatively affects a separate life history parameter. For instance, if the same gene that leads to an overproduction of a detoxifying enzyme also shunts resources away from egg production, this gene will be favored in an environment perturbed by insecticides; however, as soon as the perturbation is removed, the frequency of resistant individuals will decrease as more fecund, susceptible individuals are favored. Boivin et al. (2004) observed a correlation between hastened diapause and insecticide resistance in temperate aphids and suggested that the fitness consequences of earlier diapause in resistant individuals could affect population susceptibility. Similarly, the frequency of genes resulting in a tradeoff between resistance and life history parameters may be influenced by linkage disequilibrium. If an insecticide resistance conveying gene has a tight genetic linkage with a separate gene negatively affecting a life history parameter, the second gene will have an equal likelihood of being passed onto the next generation in environments containing insecticides. One study found that the location of two genes coding for two different detoxifying esterases were head to head on one amplicon and led the authors to infer that this linkage disequilibrium could be the cause for maintaining both genes in the population (Vaughan et al. 1997). In *M. persicae*, there exists a linkage disequilibrium

between an esterase gene and an altered gene creating a mutant AChE (target site for the

organophosphate class), and the authors hypothesize that the high frequency of resistant

populations with multiple mechanisms of resistance is due to this linkage disequilibrium

(Devonshire et al. 1998). Either of these proximate mechanisms can ultimately result in

an allocation tradeoff between life history parameters and the molecular mechanism of

resistance (Roff 2002). Proving a tradeoff exists due to reallocation of resources between

a molecular mechanisms of resistance and life history traits is difficult. However, the

technique of RNA interference has the potential to allow research into this area to begin

(see chapter 6). Understanding the costs that exist in insecticide resistant populations

compared to susceptible has important practical impactions for insecticide resistance

management.

Insecticide resistance management (IRM)

Fitness costs associated with resistance allow for the management of insecticide

resistance by favoring reversion toward susceptibility in the absence of the insecticide

(assuming that resistant alleles have not been fixed; Brown et al. 2013). The ability to

preserve classes of insecticides allows for the continued control of pest populations over

time; however, in order to manipulate these costs and utilize insecticide resistance

management methods effectively, active profiling of the pest population must occur.

Two major components of resistance management include surveying insecticide

susceptibility of the target pest and exploring molecular mechanisms that underlie any

observed resistance. Surveying insecticide susceptibility allows PMPs to choose the most

effective insecticide while avoiding those insecticides that would be ineffective (Bennett

2003, Dang et al. 2014a). Once an insecticide resistance profile of a population has been

established, an appropriate insecticide resistance management strategy can be implemented.

Many different insecticide resistance management tactics exist such as stacking (combining insecticides with more than one mode of action) active ingredients, rotating between classes or utilizing a synergist in conjunction with the current insecticide (Croft 1990, Bennett et al. 2003, Onstad 2008). The concept of rotating between chemistries with different modes of action to manage chemical resistance has been proven in bacterial (Dortch et al. 2011) and insect systems (Zhao et al. 2010). If a population of insects is resistant to pyrethroids, choosing insecticides with an alternative mode of action could favor a rapid reversion toward pyrethroid susceptibility assuming that there is a cost to maintaining such resistance. Implementation of an insecticide rotation program alternating between an organophosphate and a pyrethroid reduced the levels of resistance in populations of *Grapholita molesta*, the oriental fruit moth, in apple orchards (Kanga et al. 2003). An alternative insecticide resistance management strategy utilizes multiple classes of insecticides at one time with the idea that stacking insecticidal classes will prevent the evolution of resistance by creating a selection pressure that does not favor a single mechanism of resistance (Croft 1990, Bennett 2003, Onstad 2008). If enhanced metabolism is the mechanism of resistance, inhibiting detoxifying enzymes may be another IRM option. Insecticide synergists are compounds that inhibit insecticide metabolism and when used properly in conjunction with an insecticide, can greatly increase susceptibility of a resistant population. For example, the known P450 inhibitor piperonyl butoxide has been shown to synergize the toxicity of insecticides used to control urban pests such as cockroaches and bed bugs (Scott et al. 1990, Romero et al.

2009). Cytochrome P450sare not the only class of enzymes that can be synergized. The synergist S, S, S-tributyl phosphorotrithioate (DEF) has been shown to synergize the efficacy of organophosphates and pyrethroids in multiple dipteran pests by inhibiting activity of detoxifying esterases (Qiao et al. 1998, Zhang et al. 2007).

Goals and objectives

The goal of my research was to investigate different aspects of insecticide resistance in *C. lectularius*. In Chapter 2, I investigated the evolutionary response of multiple populations of bed bugs with varying evolutionary histories (i.e., collected from the field or maintained within the lab for many years) to combination insecticidal products that contain both a pyrethroid and a neonicotinoid (Gordon et al. 2014a). In Chapter 3, I investigated the evolutionary response of one population to selection by a neonicotinoid and the potential cross resistance between other classes. In Chapter 4, I explored fitness costs associated with selection for resistance to Temprid SC® in the three populations from Chapter 2. In Chapter 5, I documented susceptibility levels to nine commercial products containing one or two classes of insecticides (pyrethroid, neonicotinoid, pyrrole and desiccant dust) using six different populations of bed bugs. In the final chapter, I discussed the implications of my research for the development and implementation of an insecticide resistance management strategy that will delay resistance and prolong effective control of pest populations of bed bugs. I also discussed four new lines of research that could further help to characterize resistance and its consequences in the bed bug.

Chapter 2. Population variation in and selection for resistance to pyrethroid-neonicotinoid insecticides in the bed bug

Introduction

Bed bugs, *Cimex lectularius*, are hematophagous parasites that are resurging throughout the world (Doggett and Russell 2008, Mumcuoglu and Shalom 2010, Omudu and Kuse 2010, Potter et al. 2010, Bencheton et al. 2011, Kilpinen et al. 2011, Tawatsin et al. 2011). These bugs became scarce during the second part of the twentieth century, likely because they were effectively controlled by DDT and other broad-spectrum insecticides. While infestations worldwide were declining, insecticide resistance was being reported (Busvine 1958, Mallis and Miller 1964).

Insecticide resistance is a world-wide phenomenon that has been observed in hundreds of insect species of medical, urban and agricultural importance (Melander 1914, Forgash 1984, Hemmingway and Ranson 2000). Recently, pyrethroid resistance has been found in populations of bed bugs (Romero et al. 2007, Steelman et al. 2008, Yoon et al. 2008, Mamidala et al. 2011, Zhu et al. 2010) and has been implicated as one of many factors in the current resurgence of these insects. There are only a few classes of insecticides with different modes of action approved for use against bed bugs (Davies et al. 2010); thus, when pyrethroids fail, there are few options for rotation. Two classes of insecticides now commonly used to control bed bugs are pyrethroids (interfering with sodium ion channels; Soderlund and Bloomquist 1989) and neonicotinoids (acting at nicotinic acetylcholine receptors; Tomizawa and Casida 2005).

Recently, insecticidal products containing both a pyrethroid and a neonicotinoid have become available for bed bug control and are being widely used by pest

management professionals (Potter et al. 2012). Two such products are Temprid SC® (β-cyfluthrin and imidacloprid) and Transport GHP® (bifenthrin and acetamiprid). Preliminary testing of these products has been promising, but evolution of resistance is a concern, particularly given that resistance to the pyrethroid component is common.

The purpose of this study was twofold. First, to gauge susceptibility to current combination products, residual bioassays were conducted using ten populations of bed bugs. Second, to determine the evolutionary response of three of these populations to a pyrethroid/neonicotinoid combination product, two hypotheses were tested: selection imposed by Temprid SC results in decreased susceptibility to this product that also translates into cross resistance (broadest sense) to Transport GHP (a second pyrethroid/neonicotinoid combination product); and both pyrethroid and neonicotinoid resistance are increased by selection with Temprid SC.

Materials and Methods

Insects. Ten populations of bed bugs were used in this study (Table 2.4). Insects were housed in incubators away from any insecticide exposure at 26.7° C, 65 ± 5% RH, and a photoperiod of 14:10 (L:D) h. All bed bugs were fed weekly on warmed defibrinated rabbit blood (Quad Five, Ryegate, MT) through a parafilm membrane (Montes et al. 2002).

Population survey of susceptibility. A residual bioassay (Romero et al. 2007) was used to survey susceptibility of 10 bed bug populations to Temprid SC (Bayer, Research Triangle Park, NC) and Transport GHP (FMC, Philadelphia, PA). Individual wells of a 24-well cell culture plate (Costar, Corning, NY) were lined with filter paper disks (Whatman #2, cut to 1.7 cm diam.). Label rate solutions (Temprid SC 0.075% a.i

and Transport GHP 0.11% a.i.) were made by diluting the concentrated insecticide in water. Next, 50 μL of each solution was pipetted onto filter papers fitted into individual wells, and then allowed to dry completely before bugs were placed on the surface. Mortality was scored after 1, 2, 3, 7 and 14 d continuous exposure to the treated filter papers. Six groups of ten bugs (60 individuals; Table 2.4) were used for each strain of bed bugs and treatment (Temprid, Transport or water), with the exceptions of NY1, CIN1 and FF1 (all strains and treatments utilized 120 bugs) and LEX7 and LEX8 experiments using Transport (n= 59; n=51 respectively). Insects were classified as dead if they showed no movement or were unable to right themselves within 15 s of being inverted with soft forceps. Abbott's formula (Abbott 1925) was used to correct for control mortality.

    Selection experiment. A residual deposit bioassay was used to select three strains and determine susceptibility of offspring at strain specific exposure times calculated to cause 80 % mortality (ET80). Filter paper disks (Whatman #2; 4.25 cm diam.) were treated with Temprid SC (0.075%). This insecticide was applied until the paper was uniformly wetted using a fine mist sprayer (ProChemical and Dye, Somerset, MA). A second series of disks were handled similarly but treated with water to serve as a control. Disks were allowed to dry overnight. Dry disks were placed into 6-well cell culture plates (Costar; Corning, NY) with the treated surface facing up. Individuals of each strain were exposed for strain-specific exposure times (LA1 0.1 h, CIN1 1 h, NY1 19 h). Because 80% mortality was expected, large numbers of females and males from each strain were used to start selected lines (Table 2.1). After this exposure, bugs were removed from the treated surface and placed individually in wells of a 24-well plate lined with untreated

filter paper. Mortality was scored 24 h after removal from treated substrates. To serve as a control, a second group of 100 females and 40 males per strain and replicate were exposed for the same amount of time to filter papers saturated with water. Two independent pairs of selected and unselected groups were created for each laboratory population (CIN1, LA1 and NY1). After initial selection, F1 and F2 progeny were used for all subsequent experiments and received no further selection. For both selected and unselected groups, survivors were removed after 24 h and placed in feeders (75 ml plastic jars with organza covered lids; Consolidated Plastics, Stow, OH) at a sex ratio of 5 females to 2 males (to reduce detrimental effects on females caused by traumatic insemination). Parental females were allowed to oviposit on blotter paper in the feeder. Adults were transferred to a new feeder weekly leaving a group of 0 to 7 day-old eggs behind.

Offspring were reared to the adult stage using the same methods described earlier (Montes et al. 2002). These offspring were used in bioassays to measure susceptibility to Temprid SC and cross resistance to Transport GHP using the same residual assay as described above for selection with Temprid SC at the established $ET_{80}s$.

Susceptibility to β-cyfluthrin and imidacloprid. Adults from the F2 generation were used to evaluate susceptibility to imidacloprid and β-cyfluthrin (technical grade; 99.5% purity, Chem Services), the active ingredients of Temprid SC. Because numbers of bed bugs from the NY1 replicate two were adequate, the F1 generation was used. Topical bioassays were performed using doses ranging from 0.4 to 4000 ng/insect of either β-cyfluthrin or imidacloprid dissolved in acetone that was then applied to individual bed bugs with a repeating dispenser (Hamilton, Reno, NV). An aliquot (0.5

μL) of a single insecticide dose was applied to the abdomens of equal numbers of males and females housed in individual wells of a 24-well plate. For each dose, between 60 and 180 individuals were used. Bugs treated with acetone served as a control. Mortality was observed at 24 h. No control mortality was observed.

Data analysis. Correlation analysis was used to examine the relationship between Temprid SC and Transport GHP susceptibility in the ten tested populations (Analytical Software 2003). The dependence of mortality on selection was explored using a log-linear analysis with treatment (selected vs. unselected), mortality (dead vs. alive), and replicate as three dimensions of a contingency table (Sokal and Rohlf 1981). Individual replicates from the selection experiment were analyzed using χ^2 analysis (Analytical Software 2003).   Probit analysis was used for analysis of topical bioassays [AnalystSoft Inc. *BioStat v2009 - Statistical analysis program.* (2009)]. The hypotheses that test if strains are identical in terms of slope, intercept and LD$_{50}$ were evaluated using Polo Plus software (Robertson et al 2003). LD$_{50}$ values are significantly different between unselected and selected lines if the 95% confidence interval for their ratio does not include the ratio of 1.

Results

Population survey of susceptibility. Susceptibility to both Temprid SC and Transport GHP varied among populations of bed bugs (Figure 2.1). CIN10, FF1, LEX5, LEX7, LEX8, and RO1 were less susceptible to both products than CIN1, FD, LA1, and NY1 (Figure 2.1; 1d-3d). After 1 d exposure to Temprid SC, RO1, LEX5, LEX7 and LEX8 were unaffected (0% mortality), and CIN10 and FF1 had less than 5 % mortality. This same exposure time resulted in intermediate mortality for two populations (CIN1

and NY1) and 100 % mortality for LA1 and FD. However, exposure to Transport GHP

resulted in three populations with initially high mortality (CIN1, FD and LA1), five

populations with intermediate mortality (FF1, LEX5, LEX7, NY1 and RO1), and only

two populations with 0 % mortality (CIN10 and LEX8). The correlation between

susceptibility of these populations to Temprid SC and Transport GHP was initially high

at 1d (r=0.88) but progressively dropped to its lowest value at 14d (r=0.01). The initially

high correlation likely reflects the common mode of action of the active ingredients of the

two products (both contain a pyrethroid and a neonicotinoid). The lack of correlation at14

d was due to the convergence of all populations on 100% mortality with Transport GHP

(all at 100% except LEX7 which had 96.5% mortality). After 14 d exposure to Temprid

SC, CIN1, FD, and LA1 had 100% mortality with NY1 at 97.5%. At this same time, five

other populations showed intermediate levels of mortality (FF1 = 16.7%; RO1 =19.6%;

LEX5 = 32.6%; CIN10 = 57.6%; LEX7 = 61.4%). Survival of LEX8 was not affected by

Temprid SC (no mortality after 14 days).

The wide variation in susceptibility observed between populations could reflect

independence of their evolutionary history of exposure to pyrethroids, neonicotinoids, or

combinations of these active ingredients. FD and LA1 are susceptible to pyrethroids

(Romero et al. 2007). FD is a strain that has been maintained without insecticide

exposure since the early 1970s (Bartley and Harlan 1974). CIN1 was found to be highly

resistant to deltamethrin and λ-cyhalothrin (pyrethroids) when it was first established as a

laboratory colony but subsequently has reverted toward susceptibility (now only

moderately resistant; Zhu et al. 2013). Synergist and RNAi studies indicate that CIN1

has P450 mediated enhanced metabolism of pyrethroids (Romero et al. 2009; Zhu et al.

2010 and 2013). The reversion toward susceptibility could be due to a fitness cost from metabolic insecticide detoxification (Kliot and Ghanim 2012). NY1 also had an initially high level of resistance to pyrethroids (Zhu et al. 2010), which had declined somewhat before the current study (Zhu et al. 2013). NY1 was initially resistant to deltamethrin via two *kdr* target site mutations (Zhu et al. 2010) and P450-mediated enhanced metabolism (Zhu et al. 2013). All remaining populations were collected from the field after pyrethroid/neonicotinoid products became more widely used by the pest control industry for bed bugs. Thus their susceptibilities to these combination products could reflect a history of selection with either pyrethroids alone or in combination with neonicotinoids. A single action neonicotinoid product was not widely used for bed bug management before this study was initiated.

Selection experiment. Because of initial population variation in susceptibility, we exposed CIN1, LA1, and NY1 for different times to Temprid SC to impose selection (kill approximately 80%; Figure 2.2). The actual mean mortality achieved by exposing CIN1, LA1, and NY1 for 1h, 0.1h, and 19h were 83.6 (s.e.m. $\pm$ 1.0), 81.7 (s.e.m. $\pm$ 0.5), 82.4 (s.e.m. $\pm$ 3.9) % for females and 81.3 (s.e.m. $\pm$ 1.3), 85.2 (s.e.m. $\pm$ 3.8), and 93.5 (s.e.m. $\pm$ 1.3) % for males, respectively (mean of two replicates, see Table 2.1). We observed decreased susceptibility to Temprid SC within one generation in each strain. Mortality at the exposure time to the label rate material that causes an ET_{80} decreased significantly as a result of selection in CIN1 ($G^2 = 146$; df= 1; p< 0.001), LA1 (Figure 2.3; $G^2 = 101$; df= 1; p<0.001), and NY1 ($G^2 = 71$; df=1; p< 0.001; Table 2.2). When these populations were tested with an alternate combination product, Transport GHP (using the same ET_{80}s for Temprid SC) mortality decreased as a response to selection imposed with

Temprid SC for CIN1 (G^2=169; df=1; p<0.001) and LA1 (Figure 2.4; G^2= 54; df=1; p<0.001; Table 2.2) indicating cross resistance between combination products utilizing alternate active ingredients. Because there were insufficient numbers of bed bugs in the first offspring generation, NY1 was not evaluated for cross resistance between Temprid SC and Transport GHP.

Susceptibility to β-Cyfluthrin and Imidacloprid. The evolution that we observed as a result of selection with combination products could be due to changes in susceptibility to either insecticidal component or to both. Probit regression lines for all three selected strains compared to unselected strains were shifted to higher doses for β-cyfluthrin but not for imidacloprid (Figure 2.5 and Figure 2.6). All three populations showed a significant increase in the dose that kills 50% of the population (LD_{50}) after selection compared to unselected strains for β-cyfluthrin (Table 2.3). Susceptibility to imidacloprid only changed significantly in LA1 and NY1 (Table 2.3); however, LD_{50} values were relatively unaffected especially compared to β-cyfluthrin. The contrast between selected and unselected lines for all three populations significantly departed from parallelism for β-cyfluthrin but remained unchanged for imidacloprid (Table 2.3). The departure from parallelism results in the convergence of regression lines for CIN1 and NY1 and a divergence for LA1 at higher doses (Figure 2.5). This result suggests that after selection with Temprid SC the distribution of susceptibility to β-cyfluthrin changed within all populations but not to imidacloprid and further indicates that selection occurs due to the pyrethroid but not the neonicotinoid.

Discussion

There are few effective classes of insecticides available today for bed bug

management, in part because the predominant use of pyrethroid-based products has led to selection for resistance in many populations of bed bugs (Potter et al. 2010 and 2011). Phantom® (chlorfenapyr), a pyrrole insecticide acting on oxidative phosphorylation rather than sodium channels of nerve cells, killed pyrethroid-resistant bed bugs (Romero et al. 2010), but the relatively slow action of chlorfenapyr has prompted continued interest in alternatives. Dual action products containing both pyrethroids and neonicotinoids are now favored by a majority of pest management professionals (Potter et al. 2013). Given the prevalence of pyrethroid resistance in field populations, resistance to pyrethroid/neonicotinoid combination products is a concern, especially considering the diverse toxicological defenses inherent in this insect (Zhu et al. 2013). All populations of bed bugs collected in the field during the last two years were less susceptible to a pyrethroid/neonicotinoid combination than our longer maintained laboratory colonies (Table 2.4). This included one strain (LEX8) that showed no mortality after resting on freshly dried residues of Temprid SC for two weeks and three other populations with less than 50% mortality at that time. In addition to a survey of susceptibility among laboratory populations, we also investigated the potential of three of our established populations to evolve resistance to combination products under laboratory conditions.

Three populations of bed bugs (CIN1, LA1, and NY1) responded to selection with Temprid SC within one generation. This is the first laboratory documented case of decreased susceptibility as a result of selection to a pyrethroid/neonicotinoid product in bed bugs. In the absence of insecticide exposure, CIN1 and NY1 had been evolving away from the high levels of pyrethroid resistance that were initially observed (Romero et al. 2007, Zhu et al. 2010 and 2013), perhaps indicating a tradeoff between insecticide

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resistance and some unknown life history characteristics. However, if genes for pyrethroid resistance were still common in these two populations, then selection could have a large effect quickly.

Cross resistance between insecticides with the same mode of action is expected and can result in the loss of use of an entire class of insecticides (Hemmingway and Ranson 2000). Here we show that cross resistance between two pyrethroid/neonicotinoid combination products with different active constituents occurs as would be expected since collectively these constituents target the same neuronal sites (i.e., pyrethroids target sodium channels on neurons and neonicotinoids target nicotinic acetylcholine receptors). In addition, cross resistance between pyrethroids and neonicotinoids has been documented in other insects (Liu and Yue 2000, Basit et al. 2011) and is likely due to shared mechanisms of detoxification. For example, the cytochrome P450 class of enzymes is known to detoxify both pyrethroids and neonicotinoids (Scott 1999, Nauen and Denholm 2005). However, our results indicate that the change in susceptibility to Temprid SC is largely effected by a change in pyrethroid susceptibility but not neonicotinoid susceptibility.

Monitoring insecticide susceptibility and managing insecticide resistance are core principles of successful integrated pest management (Georghiou 1994, Bennett et al. 2003, Onstad 2008). The practicality of some of the principles developed in open agricultural systems are limited by zero tolerance for bed bug populations, lack of gene flow between susceptible and resistant populations, and limited number of available insecticides with different modes of action for rotation. In contrast, the return to susceptibility that has been noted in laboratory-reared populations indicates that

resistance conferring genes may not be fixed within all populations of bed bugs. Selection of control tactics based on population specific resistance profiles could make bed bug management more effective. Heterogeneous populations make it unlikely that one universally favored product will be an enduring solution. Prescription treatments would need to be implemented on a building by building basis, because within building genetic diversity is low (indicating a genetically restricted founding effect) and between building diversity is high even within a small geographic location (Booth et al. 2012). While not always economically feasible, use of alternative approaches such as whole building heat treatments or fumigation could be particularly helpful when insecticide resistance is indicated. Employing multiple tactics (heat, cold, vacuuming, bed encasement, etc.) along with vigilance and early detection may be the best way to mitigate resistance within populations of bed bugs.

Table 2.1. Percentage mortality realized when each strain was exposed to dry residues of the label rate (0.075% A.I.) of Temprid SC for an exposure time targeting 80% mortality (ET$_{80}$), and thus expected to impose strong selection

Strain	Replicate	% Mortality [a] (number treated)	
		♀[b]	♂
LA1	Rep 1	82.2(370)	81.4(70)
	Rep 2	81.2(410)	89.0(100)
CIN1	Rep 1	84.5(360)	82.5(120)
	Rep2	82.6(390)	80.0(100)
NY1	Rep 1	78.5(492)	92.2(180)
	Rep 2	86.3(490)	94.8(210)

[a] Groups of bugs were treated with the strain respective ET$_{80}$ (LA1 0.1h, CIN1 1 h, NY1 19 h; Figure 2).

[b] More females were exposed than males to achieve a sex ratio of 5:2 ♀:♂ to reduce the deleterious impact of excessive traumatic matings.

Table 2.2. Mortality of unselected and selected offspring to Temprid SC and Transport GHP using an exposure time estimated to kill 80% of the initial population prior to selection

Insecticide	Strain	Treatment	% Mortality (±s.e.m.)[a]	n[b]	G^2 (df; p-value)[c]
Temprid SC	CIN1	Unselected	69.2 (± 9.2)	120	146 (1, <0.001)[*]
	CIN1	Selected	1.6 (± 0.0)	120	
	LA1	Unselected	96.7 (±1.7)	120	101 (1, <0.001)[*]
	LA1	Selected	40.9 (±4.2)	120	
	NY1	Unselected	70.0 (±5.0)	120	71 (1; <0.001)[*]
	NY1	Selected	18.3 (±10.0)	120	
Transport GHP	CIN1	Unselected	85.0 (± 6.7)	120	169 (1; 0.001)[*]
	CIN1	Selected	10.2 (±1.9)	120	
	LA1	Unselected	100.0 (±0.0)	120	54 (1; <0.001)[*]
	LA1	Selected	71.7 (±10.0)	120	

*$P \leq 0.05$

[a] Average percent mortality and standard error calculated from each replicate of selection. Each laboratory colony was selected twice.

[b] Total individuals exposed to insecticide impregnated filter papers pooled between each replicate of selection.

[c] The dependence of mortality on selection was explored using a log-linear

analysis with treatment (selected vs. unselected), mortality (dead vs. alive), and replicate as three dimensions of a contingency table (Sokal and Rohlf 1981). Individual replicates were analyzed using χ^2 analysis (Analytical Software 2003); Figures 2.3 and 2.4).

Table 2.3. Probit analysis of topical exposure of F2 Temprid SC-selected and unselected lines to the active ingredients (A.I.), β-cyfluthrin and imidacloprid

A.I.	Strain	Treatment	n	Slope ($\pm$s.e.m.)[a]	LD_{50} ng (95% CI)[a]	LD_{50} ratio (95% CI)[b]	χ^2 (df)[a]	
						Selected/ Unselected	Goodness-of-fit[c]	Parallelism[d]
β-cyfluthrin								
	CIN1	Unselected	480	0.27 ± 0.15	0.8 (0-403,148)		2.90(2)	
		Selected	480	0.53 ± 0.10	41,518 (9,043-1,147,076)	51,458 (1,304-20.3X10^5)*	1.24(2)	3.90(1)*
	LA1	Unselected	478	1.39 ± 0.13	1.3 (1.0-1.7)		0.56(1)	
		Selected	479	1.05 ± 0.20	30.5 (4.3-1,437)	23.8 (15.1-37.4)*	2.74(2)	4.57(1)*
	NY1	Unselected	660	0.55 ± 0.16	315.0 (17.8-5,562)		7.34(2)*	
		Selected	480	0.96 ± 0.12	5,005 (3,104-9,956)	15.9 (7.3-34.5)*	2.27(1)	9.34(1)*
Imidacloprid								
	CIN1	Unselected	480	1.04 ± 0.11	103.6 (70.1-169.2)		0.35(2)	
		Selected	480	0.96 ± 0.10	71.6 (48.3-115.8)	0.7 (0.4-1.3)	1.73(2)	0.31(1)
	LA1	Unselected	480	1.24 ± 0.11	8.0 (6.0-10.8)		1.95(1)	
		Selected	480	1.11 ± 0.11	14.1 (10.2-20.4)	1.8 (1.1-2.8)*	0.01(1)	0.67(1)
	NY1	Unselected	460	0.93 ± 0.12	45.3 (23.0-73.1)		0.06 (1)	
		Selected	660	1.02 ± 0.19	126.2 (8.2-1,522)	2.8 (1.5-5.3)*	4.81(2)	0.43(1)

[a] Slope, dose that kills 50% of the population (LD_{50}), and goodness-of-fit were calculated using BioStat 2009

(AnalystSoft Inc. 2009). LD_{50} ratio and parallelism tests were performed using PoloPlus (Robertson et al. 2003).

[b] LD_{50} values are significantly different between unselected and selected lines if 1 does not fall within the 95% confidence interval for the ratio test (Robertson et al. 2003). * $P \leq 0.05$

[c] Larger values of χ^2 for goodness-of-fit indicate a poorer fit on the probit regression line. * $P \leq 0.05$

[d] Parallelism challenges the hypothesis that the slopes are identical for selected and unselected lines (Robertson et al. 2003). * $P \leq 0.05$

Table 2.4. Origins and resistance status of bed bug populations that were evaluated for their susceptibility to two pyrethroid/neonicotinoid combination products

Name	City	Collection Date	Pyrethroid Resistance[a]	Temprid SC® (n)[b]	Transport GHP® (n)[c]
CIN1	Cincinnati, OH	2005	Initially highly resistant, now moderately resistant	100 (120)	100 (120)
CIN10	Cincinnati, OH	2012	Highly resistant	57.6 (60)	100 (60)
FD	Fort Dix, NJ	<1974	Susceptible	100 (60)	100 (60)
FF1	Frankfort, KY	2012	Unknown	16.7 (120)	100 (119)
LA1	Los Angeles, CA	2007	Susceptible	100 (60)	100 (60)
LEX5	Lexington, KY	2011	Unknown	32.6 (60)	100 (60)
LEX7	Lexington, KY	2012	Highly resistant	61.4 (60)	96.5(59)
LEX8	Lexington, KY	2012	Unknown	0.0 (60)	100 (51)
NY1	New York, NY	2007	Initially highly resistant, now moderately resistant	97.5 (120)	100 (120)
RO1	Royal Oaks, MI	2012	Unknown	19.6 (60)	100 (60)

[a] Pyrethroid resistance categorization using a residual bioassay and discriminating dosage of deltamethrin (0.6%). Populations were considered susceptible if mortality was >95%, moderately resistant if mortality was <50%, and highly resistant if mortality was <5%.

[b] Percent mortality of adult bugs after 14 days of exposure to label rate Temprid.

[c] Percent mortality of adult bugs after 14 days of exposure to label rate Transport.

Figure 2.1
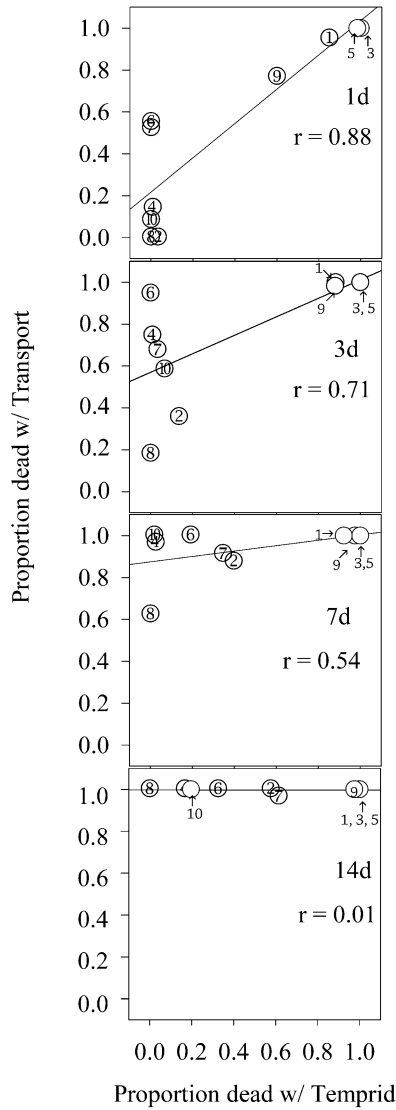
Regression between proportion of bed bugs killed by residues of Temprid SC and Transport GHP for ten populations (1 corresponds with CIN1, 2 corresponds with CIN10, 3 corresponds with FD, 4 corresponds with FF1, 5 corresponds with LA1, 6 corresponds with LEX5, 7 corresponds with LEX7, 8 corresponds with LEX8, 9 corresponds with NY1 and 10 corresponds with RO1) of bed bugs at 1 to 14d of exposure. Data shown were corrected for control mortality.

Figure 2.2
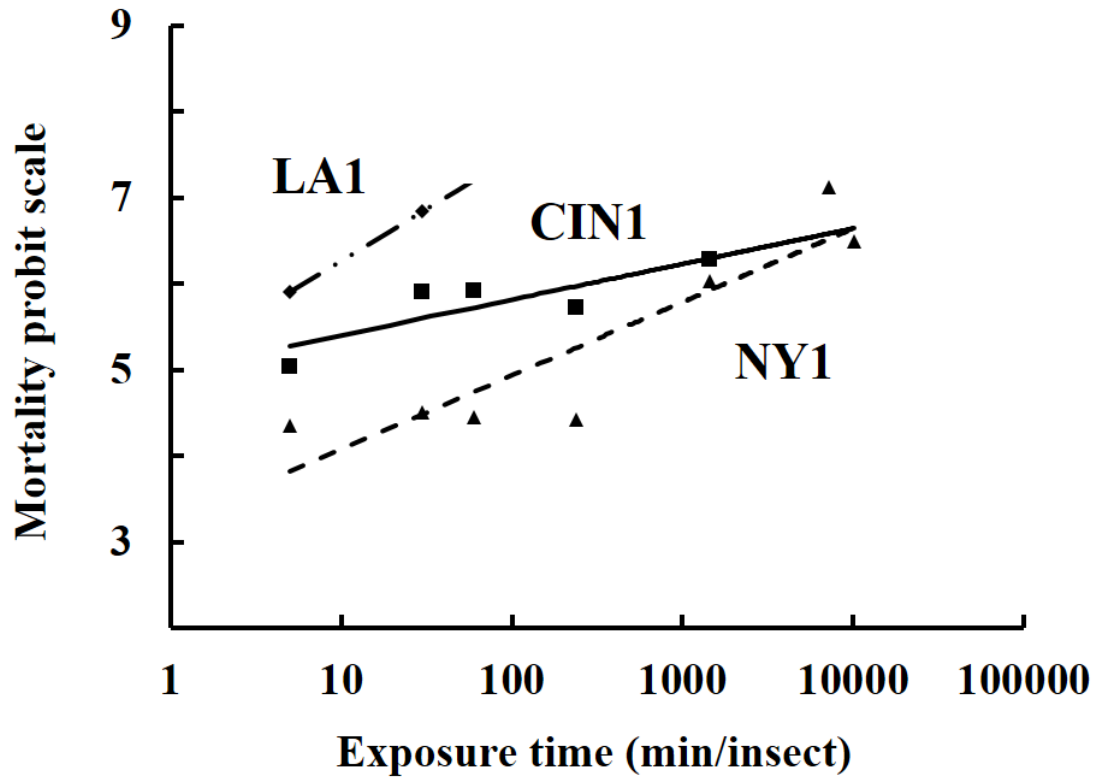
Susceptibility to Temprid between three strains of bugs. Groups of 10 bugs were exposed to filter papers saturated with label rate Temprid for different exposure times then removed. Mortality was scored after 24 h as the insect's inability to right itself after 15 s and converted to probit. No control mortality was observed.

Figure 2.3
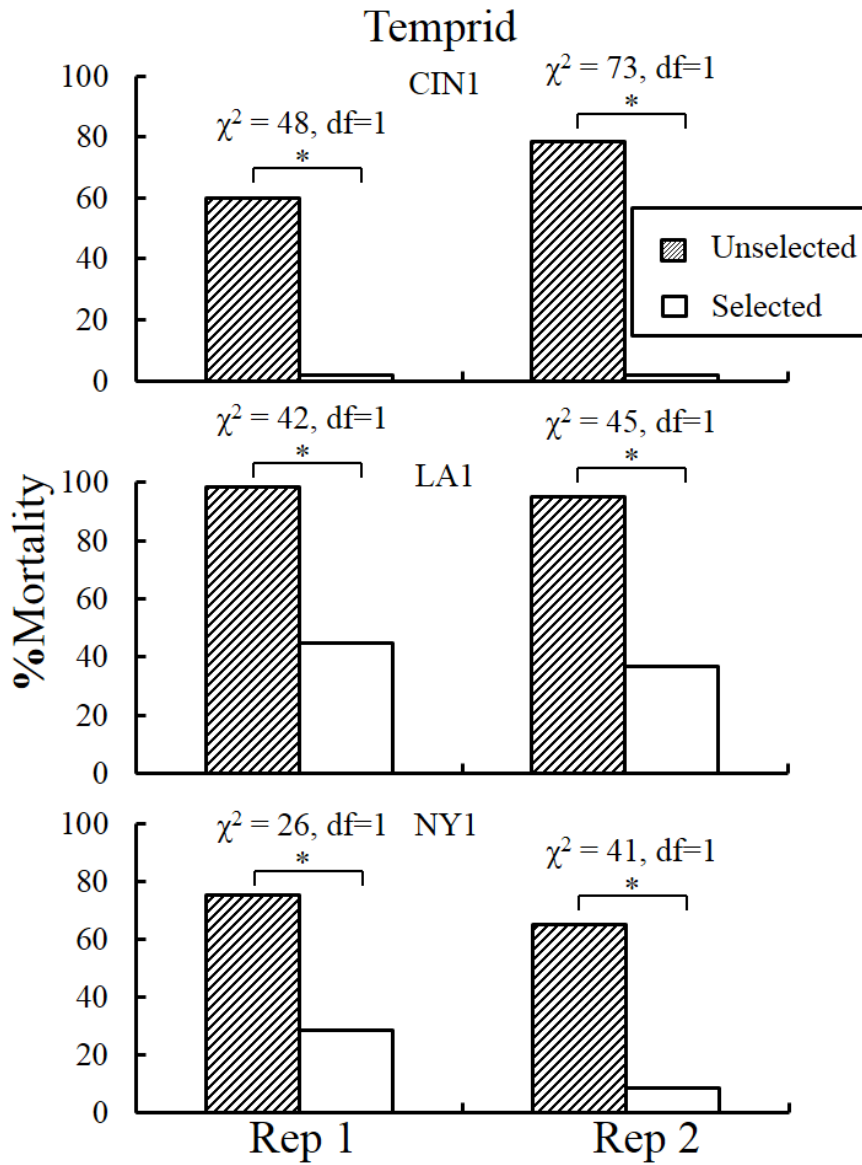
Susceptibility to Temprid SC in selected and unselected lines from CIN1, LA1, and NY1. In the parental generation adult bed bugs were exposed to residues of Temprid SC for intervals expected to kill 80% at 24 h after exposure for selected lines. Unselected lines were treated in the same way but without insecticide exposure. In each replicate of each strain there was a significant decrease (each χ^2 analysis had 1 df) in offspring mortality from the same exposure as parents received (see text for log linear analysis).

Figure 2.4
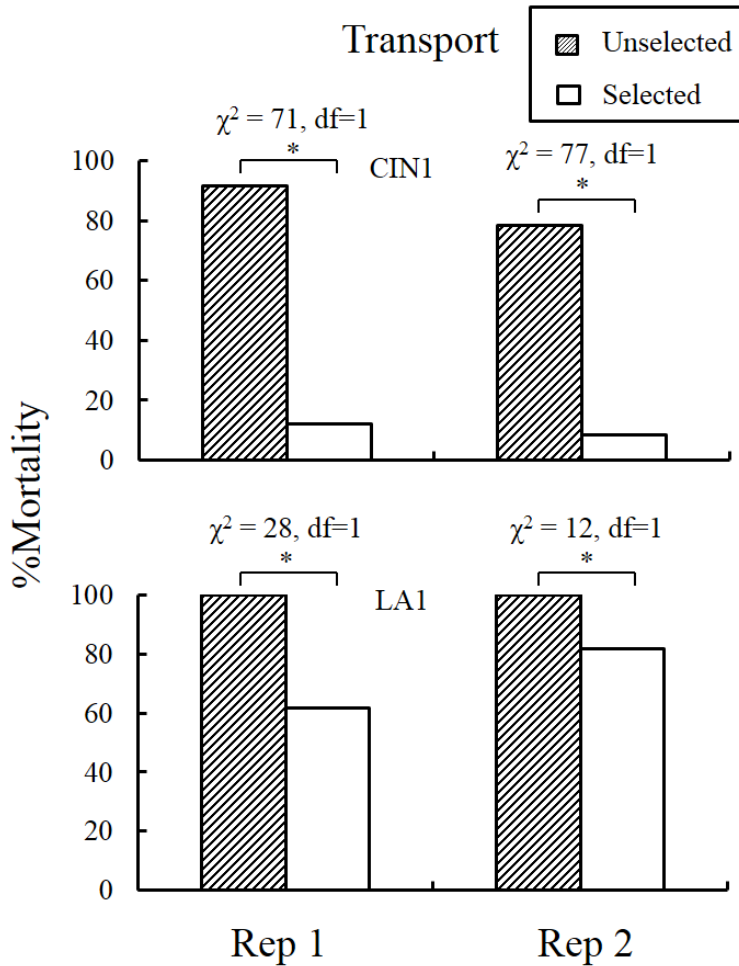
Susceptibility to Transport GHP in selected and unselected lines from CIN1 and LA1 showing cross resistance to Temprid SC. In the parental generation, adult bed bugs were exposed to residues of Temprid SC for intervals expected to kill 80% at 24 h after exposure for selected lines. Unselected lines were treated in the same way but without insecticide exposure. NY1 was not evaluated because of insufficient numbers of test insects. In each replicate of both populations, there was a significant decrease (each χ^2 analysis had 1 df) in offspring mortality from Transport GHP exposure (see text for log linear analysis).

Figure 2.5
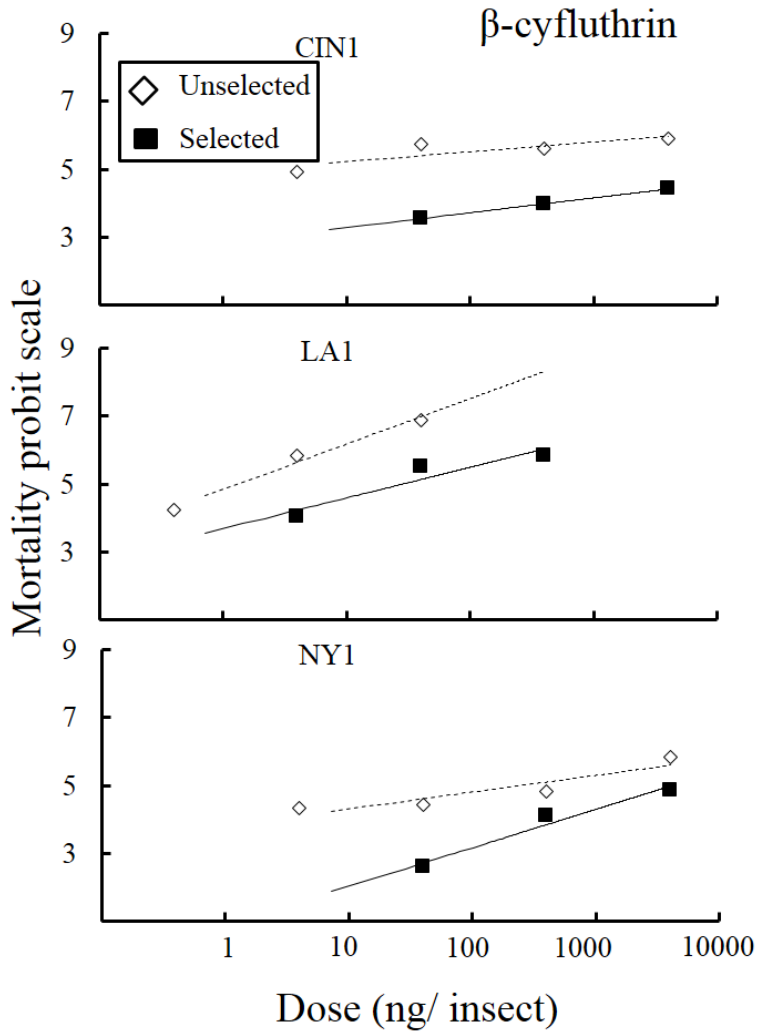
Probit regression data for the relationship between dose of β-cyfluthrin and mortality at 24 h for topical bioassays. Open diamonds and dotted lines represent unselected strains; whereas solid squares and solid lines represent selected strains.

Figure 2.6
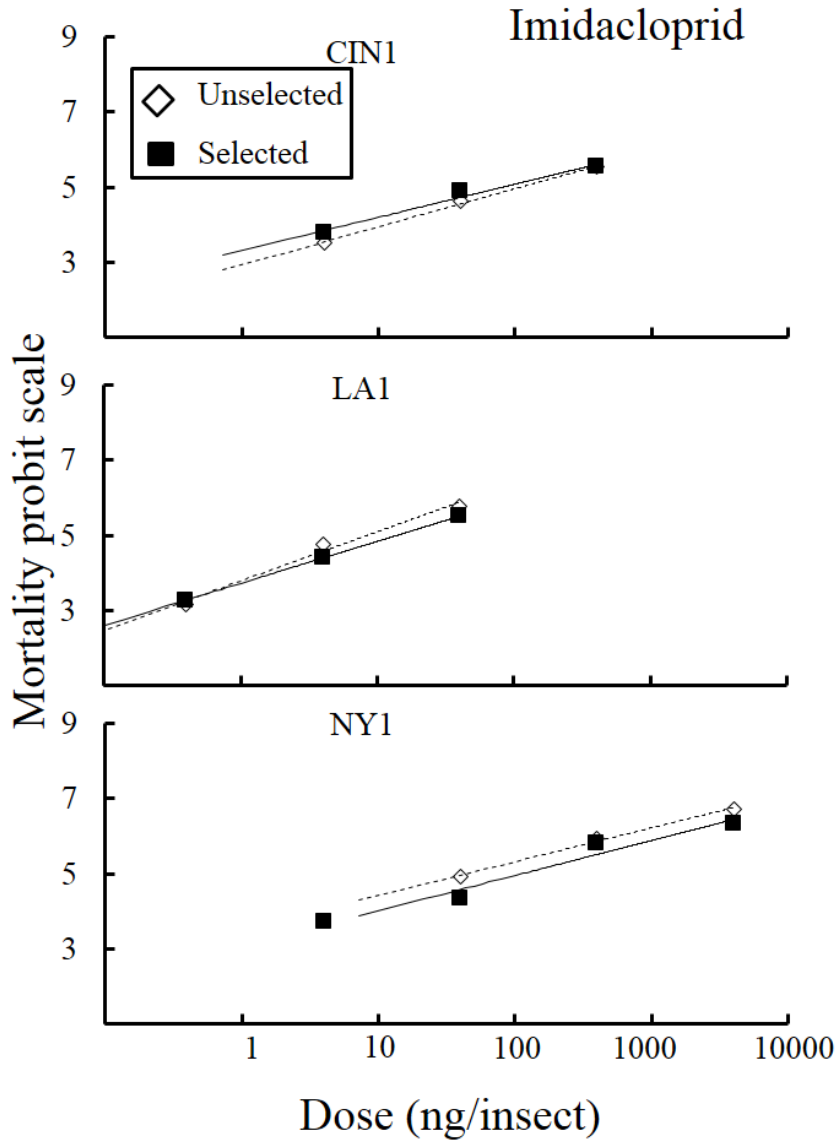
Probit regression data for the relationship between dose of imidacloprid and mortality at 24 h for topical bioassays. Open diamonds and dotted lines represent unselected strains; whereas solid squares and solid lines represent selected strains.

Chapter 3. Resistance and cross resistance to a neonicotinoid insecticide in the bed bug

Introduction

Pyrethroid resistance in the bed bug, *Cimex lectularius* is common and widespread (Romero et al. 2007, Steelman et al. 2008, Yoon et al. 2008, Zhu et al. 2010, Mamidala et al. 2011). This resistance has prompted the pest management industry to switch to products with additional or new modes of action, such as neonicotinoids, pyrroles and desiccant dusts (Romero et al. 2010, Potter et al. 2012, Gordon et al. 2014a, Potter et al. 2014). However, a more sustainable approach to bed bug control could include an effort to manage resistance before it is widely established by rotating between insecticides with alternate modes of action when pyrethroid resistance is encountered in the field (Bennett 2003, Onstad 2008).

In the United States, two neurotoxic classes of insecticides currently registered for bed bug control are the pyrethroids and the neonicotinoids. Pyrethroids kill by interfering with sodium ion channels along the nerve axon (Soderlund and Bloomquist 1989); whereas neonicotinoids bind to nicotinic acetyl choline receptors on the postsynaptic membrane of the neuron (Tomizawa and Cassida 2005). A third class of insecticides registered for bed bug control includes the pyrroles, which act by interrupting the electron transport chain in the mitochondria of insects (Hollingworth and Gadelhak 1998, Romero et al. 2010), and thus it is expected to affect all cells in addition to nerve cells. Currently, these three classes of insecticides are used in single action (commercial products containing only one active ingredients) and dual action (commercial products that utilize two classes in tandem) formulations.

Resistance to the neonicotinoid class of insecticides has been documented for multiple insects (Markussen and Kristensen et al. 2010, Basit et al. 2011, Wan et al. 2013). When the molecular mechanisms of neonicotinoid resistance have been investigated, target site mutations and changes in enzymatic detoxification have been implicated (Weill et. al 2004, Yoon et al. 2008, Markussen and Kristensen et al. 2010, Wan et al. 2013). In the cases of enhanced metabolism, the cytochrome P450 class of enzymes has been implicated, in particular, the cyp6 family within this class (Wan et al. 2013).

The current study set out to investigate three related hypotheses. First, a population's resistance to a neonicotinoid (imidacloprid) will increase with selection with this insecticide. Second, P450 mediated enhanced metabolism is the mechanism of the resultant neonicotinoid resistance. Third, an increase in resistance to imidacloprid will result in a decrease in resistance to insecticides utilizing a different mode of action, i.e., negative cross resistance.

Materials and Methods

Insects. One strain of bed bugs (CIN_{TS}) was used in this study. The strain was originally collected from Cincinnati, OH in 2005 and was resistant to pyrethroids (Romero et al. 2007, it was referred to as CIN1 at that time). However, after having been maintained in the laboratory for multiple years, this population had begun to revert back toward pyrethroid susceptibility, until it was selected in 2011 with the pyrethroid/neonicotinoid combination product Temprid SC®, resulting in increased pyrethroid resistance but not neonicotinoid resistance (Gordon et al. 2014a). Insects were housed in incubators away from any insecticide exposure at 26.7° C, 65 ± 5% RH, and a

photoperiod of 14:10 (L:D) h. All bed bugs were fed weekly on defibrinated rabbit blood warmed to 39°C (Quad Five, Ryegate, MT) through a parafilm membrane (Montes et al. 2002).

Selection experiments. A topical bioassay was used to select for imidacloprid resistance in CIN_{TS}. First, individual bugs were housed in single wells of a 24-well plate covered with parafilm and a plastic lid (the parafilm ensured that the lid would not easily fall off and that individual bed bugs could not leave their wells). Second, a dose of insecticide calculated to kill 80% of the respective generation diluted in acetone (0.5 μL) was applied to the abdomens of individual bugs using a repeating dispenser (Hamilton, Reno, NV). In addition, a group of bugs (100 females and 40 males) from the original starting population receiving no exposure to imidacloprid was treated with acetone to serve as a control. Mortality was scored after 24 h (Table 3.1). For both treated and untreated groups, survivors were removed after 24 h and placed in feeders (75 ml plastic jars with organza covered lids; Consolidated Plastics, Stow, OH) at a sex ratio of 5 females to 2 males (to reduce detrimental effects on females caused by traumatic insemination) and immediately fed. Parental females were allowed to oviposit on blotter paper in the feeder. Adults were transferred to a new feeder weekly leaving a group of 0 to 7 day-old eggs behind. Offspring were reared to the adult stage using the same methods described earlier (Montes et al. 2002) and used in subsequent experiments.

Selection occurred over the course of four generations and used two, nonsynchronous replicates; however, due to a lack of available bugs, only three generations (P, F_2 and F_3) were treated with imidacloprid (Figure 3.1). Doses of imidacloprid used in selection ranged from 112 ng/insect to 3000 ng/insect, an amount

that was adjusted based on documented susceptibility. Adult, bugs from the F_4 generation were used in all experiments.

Susceptibility to imidacloprid and β-cyfluthrin. Adult bugs from the F_4 generation were used to evaluate susceptibility to imidacloprid and β-cyfluthrin (technical grade; 99.5% purity, Chem Services). Topical bioassays were performed using doses ranging from 0.4 to 4000 ng/insect of either imidacloprid or β-cyfluthrin dissolved in acetone that was then applied to individual bed bugs. An aliquot (0.5 μL) of a single insecticide dose was applied to the abdomens of equal numbers of males and females housed in individual wells of a 24-well plate. For each dose, between 79 and 120 individuals were used (Table 3.1). Bugs treated with acetone served as a control. The number of bugs alive was observed at 24 h. Abbott's (1925) formula was used to correct for control mortality which never reached 2 %.

Susceptibility to chlorfenapyr (Phantom). Unlike all other bioassays, a residual bioassay (Romero et al. 2007) was used to determine susceptibility to chlorfenapyr in the commercial formulation Phantom SC (BASF; Research Triangle Park, NC) using F_4, adult bed bugs. Individual wells of a 24-well cell culture plate (Costar, Corning, NY) were lined with filter paper disks (Whatman #2, cut to 1.7 cm diam.). Label rate solutions were made by diluting the concentrated insecticide in water. Fifty μL of each solution was pipetted onto the filter papers fitted into the wells, and then allowed to dry completely before bugs were placed on the surface. Mortality was scored after 4, 24, 48, 96, 192 and 336 h of continuous exposure to the treated filter papers. Insects were classified as dead (including moribund) if they showed no movement or were unable to right themselves within 15 s of being inverted with soft forceps.

Synergism using PBO. Adults from the F_4 generation were used to evaluate the potential synergistic effect of the known P450 synergist piperonyl butoxide (PBO; Casida 1970) on imidacloprid susceptibility. The same topical bioassay previously described was used to obtain LD_{50} values for imidacloprid using groups of bugs pretreated with 1 µL of either 0.1 % PBO (1 µg/insect) dissolved into acetone or acetone alone. These bugs were treated individually and housed in a 24-well plate where they remained for the duration of the experiment. Once pretreated, bugs were allowed 1 hour before being treated with 0.5 µL of a dose of either imidacloprid or acetone. Doses of imidacloprid ranged from 0.4 to 4000 ng/insect dissolved in acetone. For each dose, 80 individuals from each strain were used. Bugs treated with acetone and acetone, PBO and acetone and acetone and imidacloprid served as controls. Mortality was observed at 24 h. No control mortality was observed for any control treatments except for the PBO+acetone combination, which never exceed 2 % and was corrected for using Abbott's (1925) formula.

Data analysis. Probit analysis was used for analysis of topical bioassays [AnalystSoft Inc. *BioStat v2009 - Statistical analysis program*. (2009)]. Tests comparing treatment differences in slope, intercept and LD_{50} were evaluated using Polo Plus software (Robertson et al 2003). LD_{50} values are significantly different between unselected and selected lines if the 95% confidence interval for their ratio does not include the ratio of 1.

Results

Susceptibility to imidacloprid and β-cyfluthrin. For imidacloprid, the LD_{50} increased from 15.2 to 344.0 ng/insect (Table 3.2) after three generations of selection

with this compound. At this time, the resistance ratio (LD_{50} selected/LD_{50} unselected)

was 21.7, with 95% confidence intervals not including 1.0, i.e., a significant impact on

resistance level (Table 3.2, Figure 3.2). Additionally, the slope of the probit line for bugs

selected with imidacloprid became steeper relative to those not selected suggesting that

the population became more homogenous in regards to neonicotinoid susceptibility, an

expected result as selection presumably removes those individuals that are most

susceptible to imidacloprid. The LD_{50} for β-cyfluthrin, an active ingredient that was not

used in selection, was 16.7-fold greater in the selected strain than the control strains.

However this level of resistance did not represent a significant change (95% CI for

resistance ratio includes 1.0; Figure 3.3). The slopes remained shallow and unchanged

between selected and unselected strains in regards to susceptibility to β-cyfluthrin

suggesting that both strains were heterogeneous for susceptibility to the pyrethroid

regardless of selection with a neonicotinoid.

Susceptibility to chlorfenapyr (Phantom). After three generations of selection

with imidacloprid, the LT_{50} of Phantom remained unchanged with a resistance ratio of

1.1 (Table 3.3, Figure 3.4). The slopes of both selected and unselected strains were high

compared to all other insecticides investigated suggesting that these populations are

homogenous in regards to susceptibility with Phantom. However, the high chi-square

values of both probit lines suggests that the fit of the lines are poor due to a great amount

of variance from the predicted model of the line.

Synergism using PBO. Pretreatment with PBO caused a greater synergistic

effect in the selected group compared to the unselected controls (Figure 3.5). Groups of

bed bugs that were selected with imidacloprid became 16.3-times more susceptible to

imidacloprid after exposure to PBO and were significantly different from selected bugs

not pretreated with PBO as evidenced by the 95% confident interval of this ratio not

overlapping with 1. However, pretreatment with PBO depressed resistance 2-fold in

unselected bugs (Table 3.4) but was not significantly different due to the 95% confidence

intervals overlapping with the number 1. Treatment with PBO made the slopes of both

unselected and selected strains steeper, indicating that P450s may be involved in

resistance as inhibition of this class of enzymes causes both populations to become more

homogenous in regards to imidacloprid susceptibility.

Discussion

Insecticides with neurotoxic modes of action are favored by pest management

professionals for insect management due to the quick knockdown associated with these

modes of actions. Pyrethroid and neonicotinoid classes of insecticides are two of the

neurotoxic classes currently available for managing populations of bed bugs in the United

States. Many commercial products utilize these two classes in different formulations

(Potter et al. 2012). Many pyrethroid-only products are on the market today (i.e: Suspend

SC®, Demand SC® and Tempo SC®); whereas, just one neonicotinoid-only product is

approved for bed bug control (Alpine® formulations). However, the most used

neurotoxic insecticides on the market for bed bug control are combination products that

contain both a pyrethroid and a neonicotinoid (e.g.: Transport GHP®, Temprid SC® and

Tandem SC®; Potter et al. 2012, Gordon et al. 2014a). Given that resistance to one of the

two classes within combination products is common, resistance to these dual action

insecticides in the field is a concern.

Previous work showed that populations of bed bugs evolve greater resistance to a

pyrethroid-neonicotinoid product in one generation in the laboratory (Gordon et al. 2014a). In this study, three generations of exposure to imidacloprid resulted in a 21-fold increase in LD_{50} when compared to the unselected strain. The rapid development of resistance to a neonicotinoid in the laboratory has potential ramifications for the field, considering that the rate of evolution of resistance in the bed bug is relatively fast compared to other insects (May and Dobson 1986). Given this information, pest management technicians should monitor insecticide susceptibility and may want to consider insecticide resistance management to prolong the use of effective insecticides.

As mentioned above, previous research showed that three different populations of bed bugs with varied histories of pyrethroid resistance developed increased resistance to the dual action product Temprid SC in the laboratory after one exposure (Gordon et al. 2014a). Further investigation revealed that the observed resistance to the combination product was driven by the pyrethroid component but not the neonicotinoid. In the current study, exposure to the neonicotinoid imidacloprid resulted in a significant decrease in susceptibility after three generations of selection. Unfortunately, even though the observed cross resistance between the neonicotinoid and the pyrethroid was not significant, the increased LD_{50} to β-cyfluthrin after exposure to imidacloprid suggests that rotating between these two classes will likely not manage pyrethroid resistance in this bug.

The documented neonicotinoid resistance and cross resistance to a pyrethroid is the first for a population of bed bugs. Cross resistance between these two classes has been observed in other insects (Basit et al. 2011), and cytochrome P450s have been implicated as the causative mechanism (Liu and Yue 2000). Cytochrome P450 mediated

resistance to pyrethroids has been well documented in populations of bed bugs (Mamidala et al. 2011, Zhu et al. 2012, 2013); however, no mechanism of resistance has been investigated for neonicotinoid resistance in the bed bug before this study. Results from the current study implicate P450-enhanced metabolism as one mechanism of resistance to imidacloprid and cross resistance to β-cyfluthrin. The known P450 synergist PBO increased susceptibility to imidacloprid by over 16-fold in the neonicotinoid selected strain. However, more work needs to be done to investigate the involvement of P450s in resistance to neonicotinoids and cross resistance to pyrethroids. Work from other groups found that increased expression of different enzymes in the same (*CYP6*) family of cytochrome P450s is responsible for the detoxification of insecticides in both pyrethroid and neonicotinoid classes (Markussen and Kristensen 2010, Wan et al. 2013, Zimmer et al. 2014), and thus, a P450 in that family of genes could be responsible for most of the observed resistance.

Classically, in situations where managing a pest becomes difficult due to chemical resistance, rotation between compounds having different modes of action and other chemical resistance management strategies (e.g.: stacking modes of action, adding synergists) can result in pest populations fluctuating between resistance and susceptibility to the two control methods. The concept of rotating between chemistries with different modes of action to manage chemical resistance has been proven in bacterial (Dortch et al. 2011) and insect systems (Zhao et al. 2010). Work from this study suggests that rotation between pyrethroids and neonicotinoids is not likely to be effective, but rotations with other classes of insecticides should be investigated. Multiple classes of insecticides are available for bed bug control (such as organophosphates, pyrroles and desiccant dusts)

and could be useful alternatives to traditional control using pyrethroids (Romero et al.

2010, Potter et al. 2014). Given that few classes of effective insecticides are available for

bed bug control, managing insecticide resistance may be difficult, but nonetheless a

critical component to all bed bug control programs.

Table 3.1. Realized mortality after topical treatment of imidacloprid targeting 80% mortality (LD_{80}), and thus expected to impose strong selection

Generation	Replicate	% Mortality [a] (number treated)	
		♀[b]	♂
Parental	Rep 1	81.2(500)	90.5(200)
	Rep 2	93.5(500)	91.0(200)
F_2	Rep 1	65.2(460)	61.7(240)
	Rep 2	67.2(321)	55.0(160)
F_3	Rep 1	78.5(400)	78.9(180)
	Rep 2	82.9(310)	88.0(200)

[a] Groups of bugs were initially treated with the an LD_{80} (Gordon et al. 2014a).

[b] More females were exposed than males to achieve a sex ratio of 5:2 ♀:♂ to reduce the deleterious impact of excessive traumatic matings.

Table 3. 2. Analysis of mortality of bed bugs from the F$_4$ generation treated topically with either imidacloprid or β-cyfluthrin

Selection	Treatment	Slope (±SE)[a]	LD$_{50}$ ng/insect (95% CI)[a]	LD$_{50}$ ratio (95% CI)[b]	χ^2 (df)[a]
				Selected/ Unselected	Goodness- of-fit
Imidacloprid					
	Unselected	0.7 (±0.1)	15.2 (3.2-35.5)	21.7(7.0-67.6)[*]	1.1 (3)
	Selected	1.1(±0.2)	344.0 (128.4-778.4)		3.0 (3)
β-cyfluthrin					
	Unselected	0.4 (±0.1)	2.1×10^4 (6.5×10^4- 3.2×10^5)	16.7(0.25-1132.4)	3.8 (3)
	Selected	0.4 (±0.1)	3.5×10^5 (3.1×10^5- 3.0×10^9)		1.0 (3)

[a] Slope, dose that kills 50% of the population (LD$_{50}$), and goodness-of-fit were calculated using BioStat (AnalystSoft Inc. 2009).

[b] LD$_{50}$ ratio tests were performed using PoloPlus (Robertson et al. 2003).LD$_{50}$ values are significantly different between unselected and selected lines if 1 does not fall within the 95% confidence interval for the ratio test (Robertson et al. 2003). [*]$P \leq 0.05$

Table 3.3. Analysis of exposure time to Phantom using imidacloprid selected and unselected bugs from the F4 generation

Treatment	Slope ($\pm$SE)[a]	LD$_{50}$ ng/insect (95% CI)[a]	LD$_{50}$ ratio (95% CI)[b]	χ^2 (df)[a]
			Selected/Unselected	Goodness-of-fit
Unselected	4.8 ($\pm$0.4)	92.9 (84.2-101.8)		2.3×10^7 (4)[*]
Selected	4.7 ($\pm$0.4)	104.3 (94.5-114.0)	1.1(1.0-1.3)	6.2×10^7 (4)[*]

[a] Slope, dose that kills 50% of the population (LD$_{50}$), and goodness-of-fit were calculated using BioStat (AnalystSoft Inc. 2009). [*]$P \leq 0.05$

[b] LD$_{50}$ ratio and parallelism tests were performed using PoloPlus (Robertson et al. 2003). LD$_{50}$ values are significantly different between unselected and selected lines if 1 does not fall within the 95% confidence interval for the ratio test (Robertson et al. 2003).

Table 3.4. Analysis of mortality of imidacloprid after pretreatment with either piperonyl butoxide (PBO) or acetone using F_4 imidacloprid-selected and unselected bed bugs

Strain	Treatment	Slope (±SE)[a]	LD$_{50}$ ng/insect (95% CI)[a]	Synergism ratio (95% CI)[b]	χ^2 (df)[a]
Unselected				Selected/ Unselected	Goodness-of-fit
	Imidacloprid	0.7 (±0.1)	15.2 (3.2-35.5)		1.1 (3)
	Imidacloprid PBO	2.2(±0.2)	8.0 (6.7-9.6)	2.0 (0.7-6.0)	1.8 (3)
Selected					
	Imidacloprid	1.1(±0.2)	344.0 (128.4-778.4)		3.0 (3)
	Imidacloprid PBO	1.5(±0.2)	21.0 (10.3-47.0)	16.3(11.2-23.8)[*]	4.7 (3)

[a] Slope, dose that kills 50% of the population (LD$_{50}$), and goodness-of-fit were calculated using BioStat (AnalystSoft Inc. 2009).

[b] Synergism ratio tests were performed using PoloPlus (Robertson et al. 2003). Synergism ratio compares LD$_{50}$ values between pretreatments of either PBO or acetone. Vales are significantly different between unselected and selected lines if 1 does not fall within the 95% confidence interval for the ratio test (Robertson et al. 2003).

[*]$P \leq 0.05$

Figure 3.1
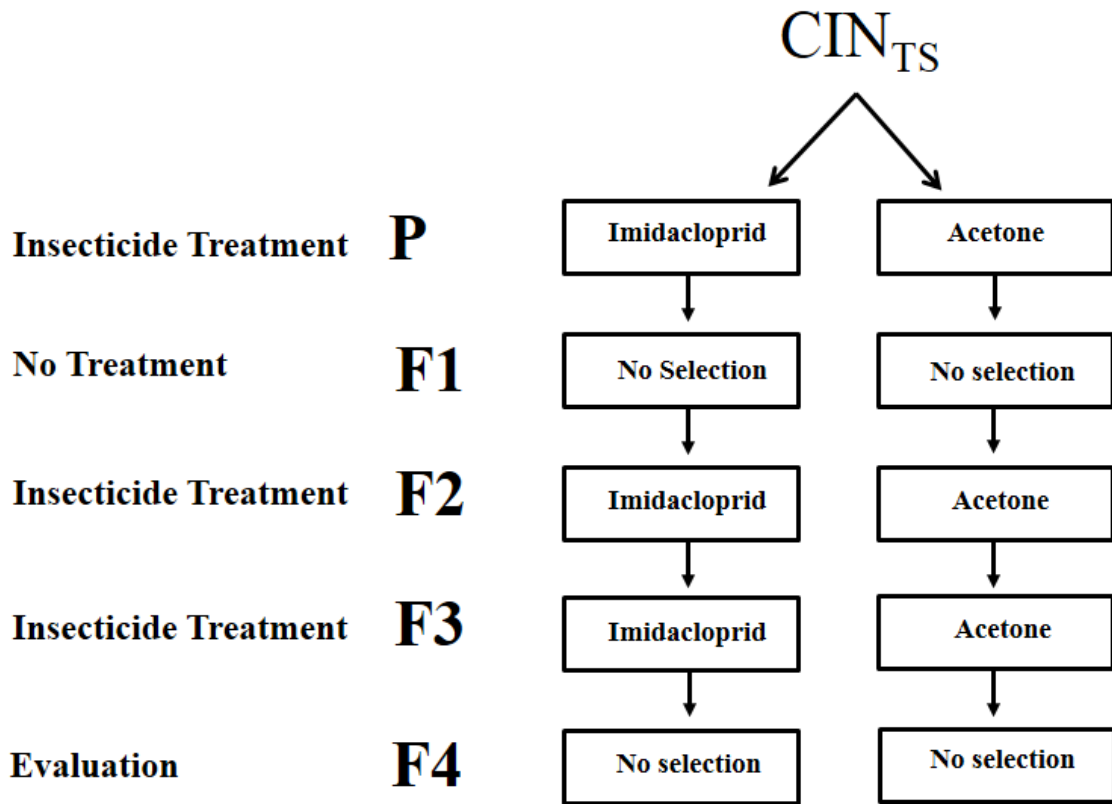
Experimental design for selection experiment. For each insecticide treatment, one dose of imidacloprid in solution with acetone calculated to kill 80% of the respective generation was topically applied to the abdomens of bed bugs. The selection experiment was performed twice (two replicates). Replicates were not synchronous and separated by weeks.

Figure 3.2
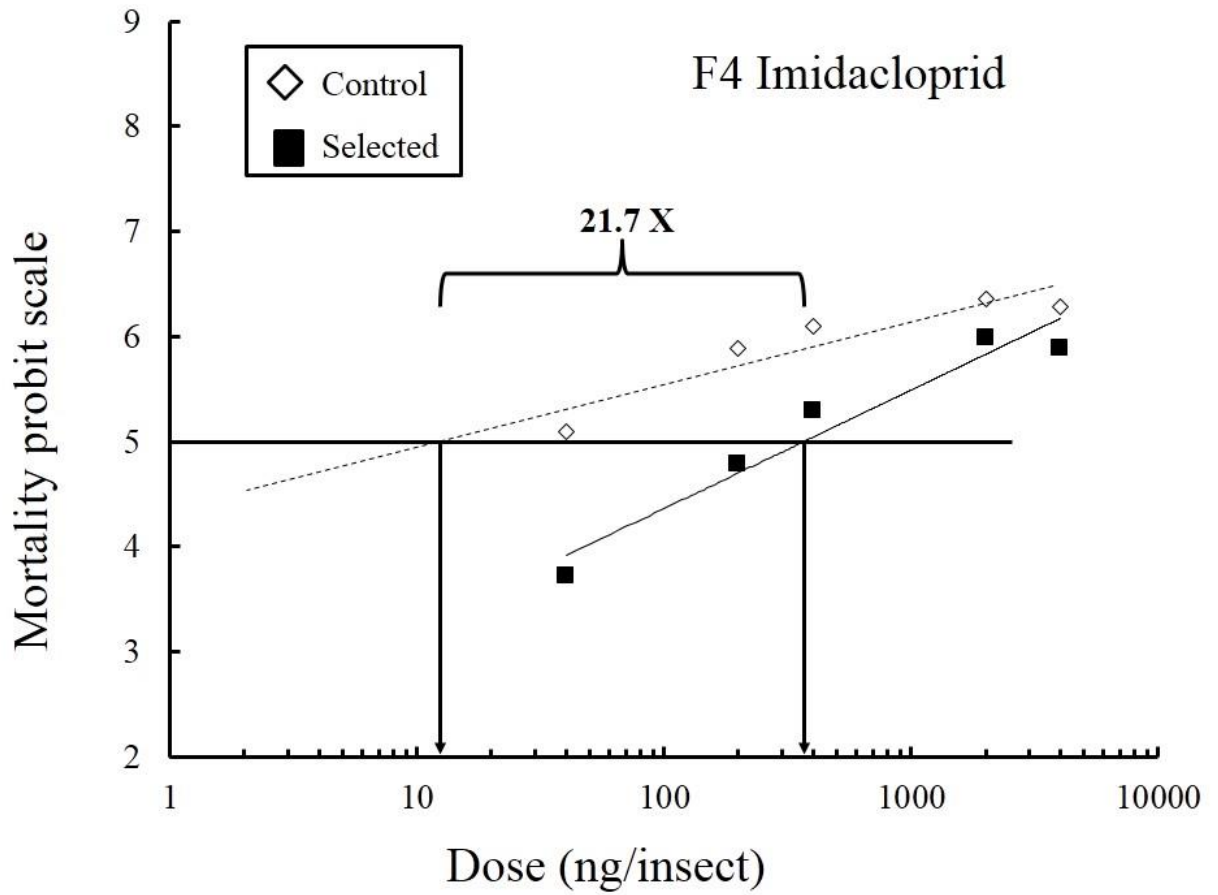
Probit regression data for the relationship between dose of imidacloprid and mortality at 24 h for topical bioassays after three generations of selection. Open diamonds and dotted lines represent unselected strains; whereas solid squares and solid lines represent selected strains.

Figure 3.3
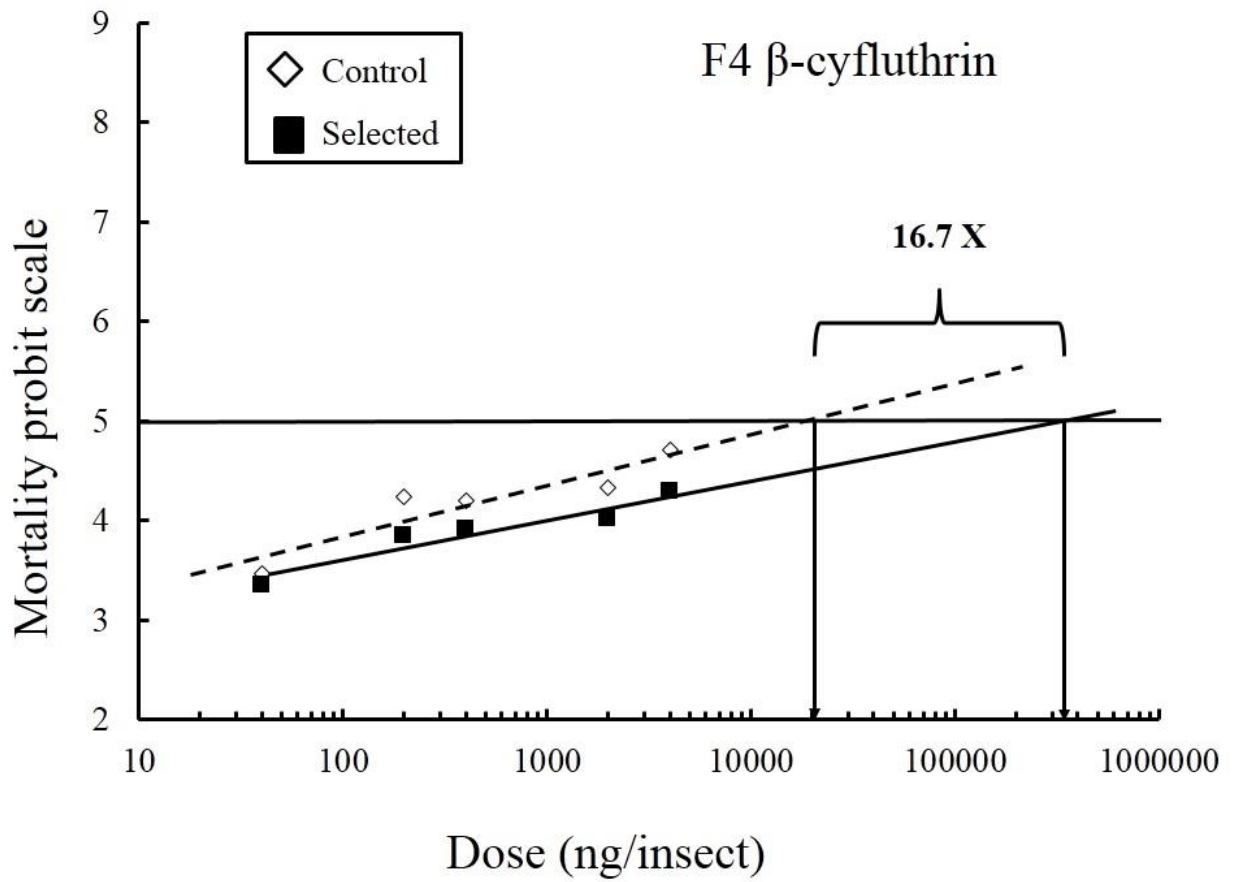
Probit regression data for the relationship between dose of β-cyfluthrin and mortality at 24 h for topical bioassays after three generations of selection. Open diamonds and dotted lines represent unselected strains; whereas solid squares and solid lines represent selected strains.

Figure 3.4
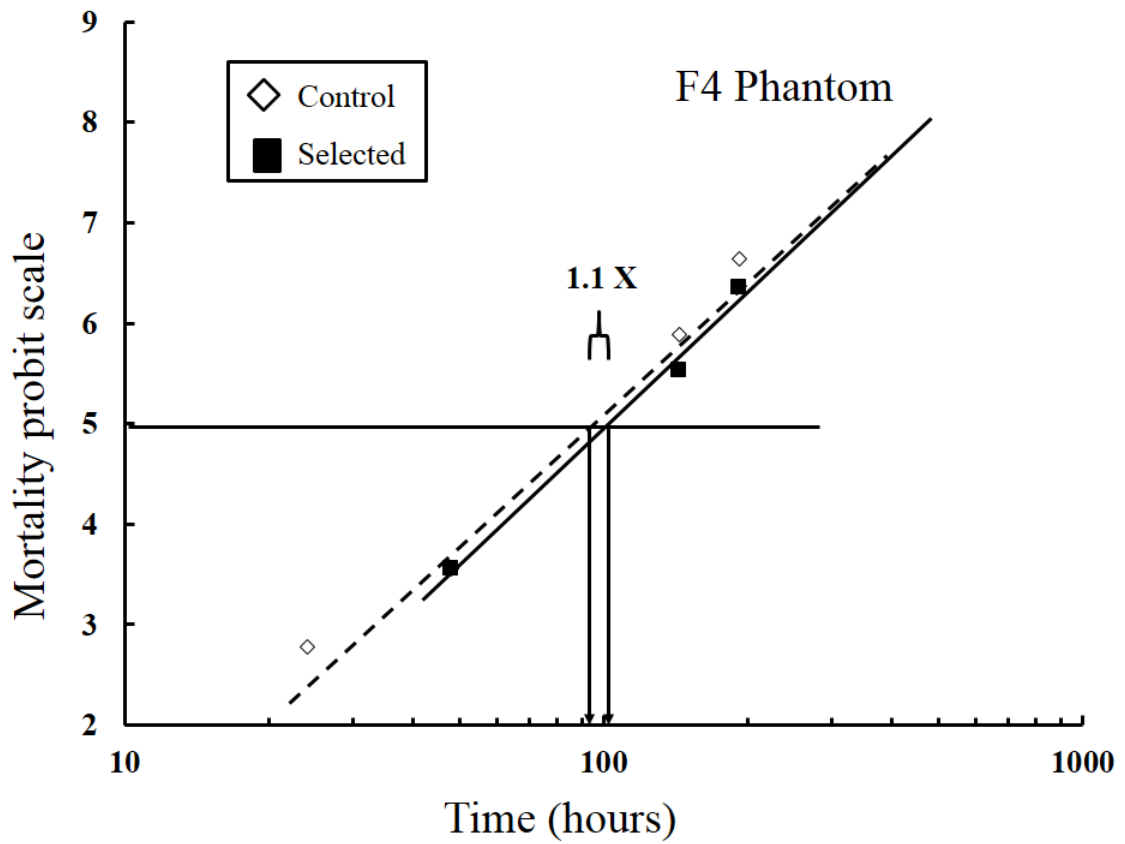
Probit regression data for the relationship between exposure time to Phantom SC and time. Open diamonds and dotted lines represent unselected strains; whereas solid squares and solid lines represent selected strains.

Figure 3.5
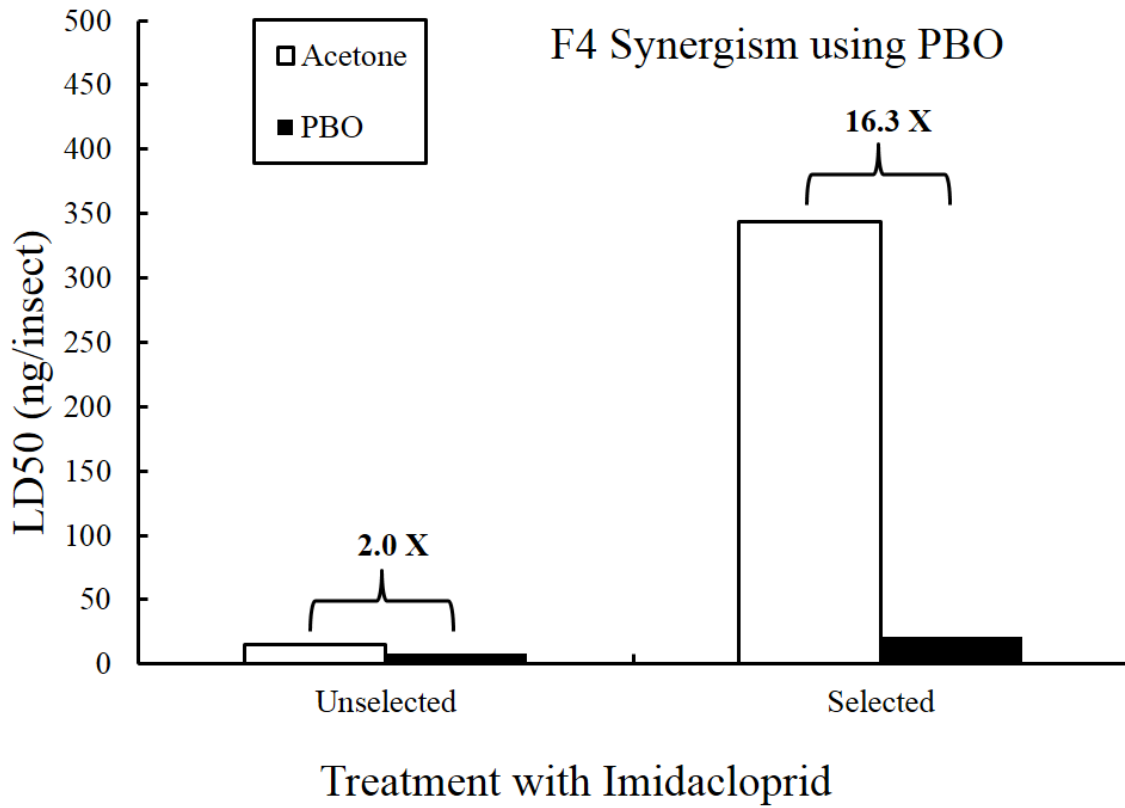
Synergistic effect of piperonyl butoxide (PBO) on susceptibility to imidacloprid.

Chapter 4. Life history tradeoffs associated with insecticide resistance in the bed bug

Introduction

Adaptation to a new environmental stress is often associated with an alteration of one or more life history parameters (Stearns 1989, Roff 2002). Ultimately, these tradeoffs may be the result of a physiological constraint, such as shunting resources into survival in the new environment that can no longer be used for egg production or rapid development. More proximately, a genetic correlation between life history traits may explain the intergenerational response. In the case of insecticide resistance, increased production of enzymes leading to insecticide detoxification or increased production of cuticular components that reduce penetration of the toxicant may have correlated effects on life history characters. Among various species, reduced longevity, delayed maturation and decreased egg production have been observed to accompany insecticide resistance (Carriere et al. 1994, Liu and Han 2006, Pereira et al. 2011, Kliot and Ghanim 2012, Martins et al. 2012, Otali et al. 2014). The net result of the adaptations to insecticide exposure is enhanced fitness with insecticide perturbation but decreased fitness in an insecticide-free environment.

Such tradeoffs open the possibility of insecticide resistance management by rotation between compounds with different modes of action (and hence different physiological costs) or to non-insecticidal control tactics. When there are tradeoffs associated with resistance in environments away from the insecticide, by removing the insecticide, the susceptible individuals should be favored, and the population should revert toward susceptible (Croft 1990, Bennett et al. 2003, Onstad 2008). If no costs

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existed for maintaining resistance, insecticide resistance management would not be effective (Brown et al. 2013).

In the past ten to fifteen years there has been a resurgence of pyrethroid resistant populations of *Cimex lectularius*, the bed bug (Romero et al. 2007, Steelman et al. 2008, Yoon et al. 2008, Mamidala et al. 2011). Alleles for pyrethroid resistance are widespread in the United States, Australia and presumably elsewhere (Zhu et al. 2010, Dang et al. 2014b). Resistance to pyrethroid-only insecticides has prompted a shift by pest management professionals (PMPs) to commercial insecticide products containing both a pyrethroid and a neonicotinoid (Potter et al. 2012, Gordon et al. 2014a). These two classes of insecticides act at different target sites on the insect neuron (Soderlund and Bloomquist 1989, Tomizawa and Cassida 2005).

Our previous work investigating the evolutionary response of multiple populations of bed bugs to these combination products showed that resistance began to evolve in one generation in the laboratory (Gordon et al. 2014a). This rapid evolution under laboratory conditions gave us an opportunity to explore the hypothesis that life history costs would be associated with decreased susceptibility to the combination insecticide.

Material and Methods

Insects. Three strains of bed bugs were used for this study. The LA1 strain was collected from Los Angeles in 2007 and was susceptible to pyrethroids (Romero et al. 2007). The strain CIN1 was originally collected from Cincinnati, OH in 2005 and was resistant to pyrethroids (Romero et al. 2007). Subsequently, its originally high level of pyrethroid resistance has declined but not to the level of the susceptible colony LA1 (Zhu

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et al. 2013). The NY1 strain was collected from New York City, NY in 2007 and was resistant to pyrethroids (Zhu et al. 2010). However, a reversion toward susceptibility has also been recorded for this strain, though not to the degree of CIN1 (Zhu et al. 2013). For each strain, two samples of bugs were selected overtime, and two separate lineages of selected and unselected strains were initiated for LA1, CIN1 and NY1 by exposing these strains to residual deposits of the pyrethroid/neonicotinoid combination product Temprid SC® for a time calculated to kill 80 % of the population (ET_{80}) at the label rate (Gordon et al. 2014a). Bugs from the F_1 generation were used to evaluate susceptibility and establish an F_2 generation (Figure 4.1). Insects were housed in incubators away from any insecticide exposure at 26.7° C, 65 ± 5% RH, and a photoperiod of 14:10 (L:D) h. All bed bugs were fed weekly on defibrinated rabbit blood (Quad Five, Ryegate, MT) warmed to 39°C with a circulating water bath (Montes et al. 2002).

   Insecticide bioassays.   Susceptibility to the combination product was followed through the F_3 generation using the residual bioassay described by Gordon et al. (2014a) to ensure that the change in resistance recorded was evidence of an evolutionary response and not the result of a different mechanism (i.e., maternal effect). Filter paper disks (Whatman #2; 4.25 cm diam.) were treated with Temprid SC at the labeled concentration (0.075% total active ingredients or 0.05% imidacloprid and 0.025% β-cyfluthrin). This insecticide was applied until the paper was uniformly wetted using a fine mist sprayer (ProChemical and Dye, Somerset, MA). A second series of disks were handled similarly but treated with water to serve as a control. Disks were allowed to dry overnight. Dry disks were placed into 6-well cell culture plates (Costar; Corning, NY) with the treated surface facing up. Because of major differences between starting populations in

susceptibility to Temprid SC, groups of 10 bugs from each strain were exposed to previously determined, strain-specific exposure times calculated to kill 80% of the population (LA1 0.1 h, CIN1 1 h, NY1 19 h; Gordon et al. 2014a; Figure 4.2). After this exposure, bugs were removed from the treated surface and placed individually in wells of a 24-well plate lined with untreated filter paper. Mortality was scored 24 h after removal from treated substrates.

Life history variables. Life history data was collected for each strain and treatment. A group of 20 eggs was gathered within a 24 hour period from three to 12 females and maintained within a small petri dish (5.1 cm diameter) lined with black filter paper (Table 4.1). These eggs were allowed to eclose and the resulting individuals were fed weekly for the duration of their lives. Adult offspring were allowed to mate *ad libitum.* The percentage of eggs hatching, the percentage of individuals reaching the adult stage, the number of eggs per female, the sex ratio at maturity, the duration of oviposition, and time course from egg to egg in the two generations was recorded weekly. In addition, this information was used to generate weekly survival (l_x) and birth curves (m_x). These observed values were used to calculate survival (l_x; female specific longevity could not be calculated due to a lack of information about sex until eclosion to the adult stage) and birth rates, in this case oviposition rates (m_x), and then net reproductive rate (R_o). Because sex determination was only made during the adult stage, estimates of l_x, m_x, and R_o were based on the assumption of a 1:1 sex ratio of eggs laid by adult females. The F_2 generation for each replicate was followed for up to 71 weeks when the last individual died. Within strains, samples of replicates 1 and 2 were separated in time by 2 to 4 weeks.

Data analysis. A general linear model was used to investigate the effects of strain, treatment and replicate within strain on the hatch rate of eggs laid by F_1 mothers, the per cent of those eggs that reach adulthood, the mean reproductive rate assuming a 1:1 sex ratio of eggs laid by F_2 females, the number of eggs per female, the observed sex ratios and the mean oviposition duration (Systat Software 2008). Net reproductive rate (R_o) was calculated as the sum of l_x*m_x for each weekly interval for each replicate with the assumption of a 1:1 sex ratio of eggs laid by F_2 females.

Results

Insecticide bioassays. Susceptibility to Temprid SC remained relatively consistent through the F3 generation for both selected and unselected groups (Figure 4.2). In all strains, those lineages that had been selected by exposure to residues of Temprid SC in the parental generation remained more resistant in the F1, F2, and F3 generations than their counterparts not exposed to insecticide.

Life history variables. Previous treatment with Temprid SC significantly affected some, but not all, measured parameters. Longevity, percent hatch and percent reaching the adult stage were not affected by the history of selection (Figures 4.3, 4.4 and 4.5). The longest lived individual was a male from the NY1 selected line that lived for 71 weeks. Reproductive rate (R_o) decreased in all groups exposed to insecticide relative to their unselected counterparts and there was a significant effect of strain on this characteristic (Figure 4.6). The average number of eggs per female was less in all populations with a history of insecticide exposure in the laboratory compared to those not treated (Figure 4.7). The sex ratio at maturity was biased towards females in unselected lines and biased towards males in selected lines (Figure 4.8). Additionally, the total time

of oviposition duration from the first egg laid to the last egg laid was significantly shorter

for those groups exposed to insecticides compared to those not exposed (Figure 4.9).

Discussion

Three different strains of bed bugs with different evolutionary histories incurred

significant life history costs after selection with the pyrethroid/neonicotinoid combination

product Temprid SC. In all three strains, we recorded a decrease in fecundity, longevity,

R_o, oviposition duration and percent of females in selected lineages relative to unselected

lines. In general, the costs of resistance increased as the level of resistance increased (i.e.,

NY1> CIN1> LA1). Additionally, there was a significant effect of strain on R_o

suggesting that as resistance increased the strain's R_o decreased. Similar tradeoffs

between insecticide resistance and life history parameters have been recorded in other

insect pests as well (Carriere et al. 1994, Mebrahtu et al. 1997, Liu and Han 2006, Pereira

et al. 2011, Martins et al. 2012, Kliot and Ghanim 2012, Otali et al. 2014).

In this system, one possible mechanism of the observed costs could be a tradeoff

between production of detoxifying enzymes and allocation of resources for fecundity. A

previously published study investigating the molecular mechanisms of resistance in the

CIN1 selected strain found that four cytochrome P450s and one carboxylesterase was

significantly over expressed compared to the CIN1 unselected line (Zhu et al. 2013).

Whereas molecular mechanisms of resistance were not investigated for the LA1 and NY1

selected strains, increased detoxification is likely at least one mechanism of resistance in

the LA1 and NY1 selected strains as well. Research investigating mechanisms of

resistance in many populations of *C. lectularius* found that the P450 class of enzymes

frequently confers a level of resistance to pyrethroid insecticides (Romero et al. 2009,

Adelman et al. 2011, Bai et al. 2011, Mamidala et al. 2012, Zhu et al. 2012 and 2013).

Given this information, resources may be shunted from fecundity to the production of detoxifying enzymes. Further experiments involving the use of RNA interference could be used to elucidate the molecular mechanism of the observed costs.

A similar study investigating population growth potential of different strains of bed bugs with different insecticide susceptibility profiles also found that insecticide resistant strains had fitness costs compared to more susceptible strains (Polanco et al. 2011). However, the present study is the first time that fitness costs in multiple populations of *C. lectularius* can be attributed to the evolution of insecticide resistance. In the Polanco et al. (2011) study, three different strains of bed bugs with different evolutionary histories and susceptibility profiles were compared. Thus, any observed costs could be due to the different origins of the populations and not necessarily insecticide resistance. However, the significant effect treatment had on several life history parameters investigated in the current study suggests that previous exposure to residues of an insecticide resulted in decreased insecticide susceptibility and increased fitness costs away from the insecticide compared to the same populations never exposed to the insecticide. Strain was also found to have a significant effect on R_o and eggs per female, which could be explained by different evolutionary histories or provide further evidence that insecticide resistance carries fitness costs (i.e., NY1> CIN1> LA1).

The evolution of resistance in all three strains of bed bugs was rapid (Gordon et al. 2014a) especially relative to other insects (May and Dobson 1986); however, given that the R_o ratios of selected and unselected lines is 0.70, 0.42 and 0.57 for LA1, CIN1 and NY1, respectively, a reversion to susceptibility should occur rapidly in environments

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no longer containing insecticides. To further investigate the idea of reversion, an estimate of the time course to 50 and 90% recovery of pre-insecticide selection levels of susceptibility was modeled (May and Dobson 1986) where T_r denotes the time required to reach a significant degree of susceptibility, T_g denotes generation time, p_f denotes the proportion of the population required to be susceptible, p_0 denotes the initial proportion susceptible, w_s denotes the fitness of the susceptible and w_r denotes the fitness of the resistant.

$$T_r \cong T_g [\frac{\ln\left(\frac{p_f}{p_0}\right)}{\ln\left(\frac{w_s}{w_r}\right)}]$$

Generation time was calculated by taking the summation of l_x*m_x*x divided by the summation of l_x*m_x (Price 1975), and the R_os for each treatment and strain were used for the fitness variables w_s and w_r. Results from the model found that the generations required for a strain to contain 50% susceptible individuals was 3.03, 4.88 and 0.71 for LA1, CIN1 and NY1, respectively. Additionally, the same model predicted that the generations required for a strain to contain 90% susceptible individuals was 6.51, 5.95 and 2.06 for LA1, CIN1 and NY1, respectively. Actual results from monitoring susceptibility away from insecticidal exposure contradicted the predications of this model (Figure 4.2). The disparity between the model's predictions and actual results is likely because resistance in populations of bed bugs violates the assumption that resistance involves only one locus and two alleles (May and Dobson 1986). Research investigating the molecular mechanisms of insecticide resistance in the bed bug supports this idea, because resistance in this pest is often polygenic (Zhu et al. 2010, Adelman et al. 2011, Bai et al. 2011, Mamidala et al. 2012, Zhu et al. 2013). Alternatively, the model's

predictions may have been incorrect, because the differences in fitness between selected and unselected individuals is an unknown (and perhaps unlikely) artifact of selection bias rather than a difference in insecticide susceptibility.

Currently, the combination pyrethroid/neonicotinoid products are some of the most effective choices for control in the field (Potter et al. 2012). In theory, rotation to products utilizing alternative modes of actions could stop or even reverse resistance (Carriere et al. 1994, Bennett et al. 2003 and Onstad 2008) assuming that alleles for pyrethroid susceptibility still exist in the population. For bed bugs, effective alternative insecticides for rotation might include chlorfenapyr, a disruptor of mitochondrial oxidative phosphorylation, and silica gel, a desiccant dust that efficiently removes cuticular waxes (Romero et al. 2010, Potter et al. 2014). Integrated pest management utilizing a variety of non-pesticidal methods combined with rotation of chemistries will be vital for the continued management of *C. lectularius.*

Table 4.1. Number of female bed bugs from the F$_1$ generation used to collect 20 eggs from each replicate and sample

Strain	Treatment[a]	Replicate 1[b]		Replicate 2	
		a[c]	b	a	b
LA1					
	Unselected	9	6	12	6
	Selected	6	3	11	6
CIN1					
	Unselected	7	9	12	6
	Selected	11	9	11	7
NY1					
	Unselected	9	9	9	6
	Selected	9	10	8	7

[a] Selected individuals were exposed to label rate Temprid SC for a time expected to kill 80 % of the populations (Gordon et al. 2014a).

[b] Replicate refers to the asynchronous exposure of two different groups of each strain to Temprid SC.

[c] Sample refers to the asynchronous collection of 20 eggs from each replicate, treatment and strain.

Figure 4.1
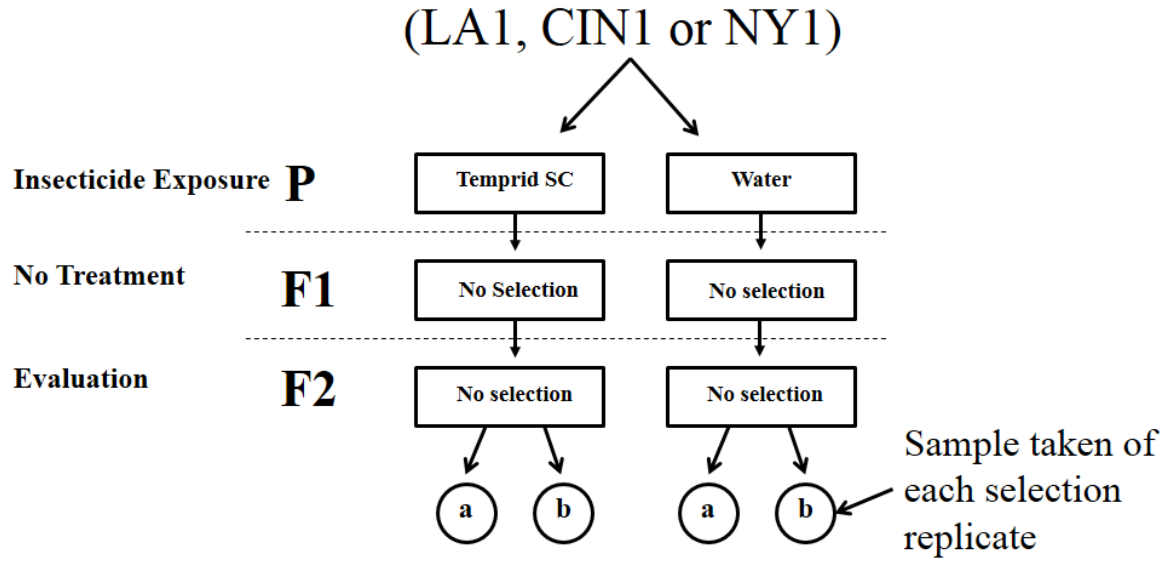
Experimental design for selection experiment. Bugs were exposed to label rate Temprid SC® for a time calculated to kill 80% of the respective strain of bed bugs. The selection experiment was performed twice (two replicates) per strain. Replicates were not synchronous and separated by weeks.

Figure 4.2
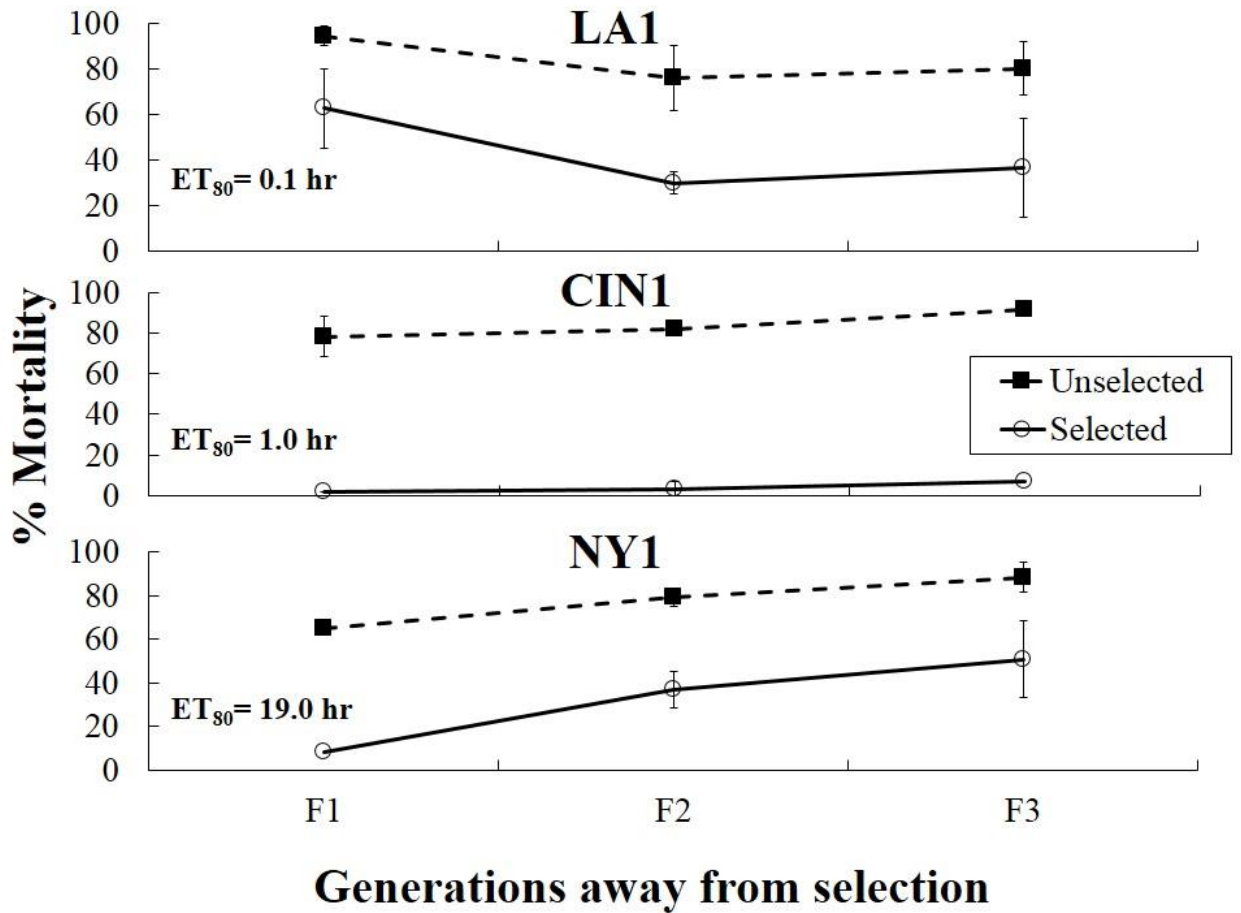
Mortality of all strains through the F3 generation. Susceptibility of strains was evaluated by exposing groups of bugs to residual deposits of Temprid SC following the protocol of Gordon et al. (2014a). Individuals from each strain were exposed for strain-specific exposure times (LA1 0.1 h, CIN1 1 h, NY1 19 h) calculated to kill 80 per cent of the population (ET_{80}). Open diamonds and dotted lines represent unselected strains; whereas solid squares and solid lines represent selected strains.

Figure 4.3
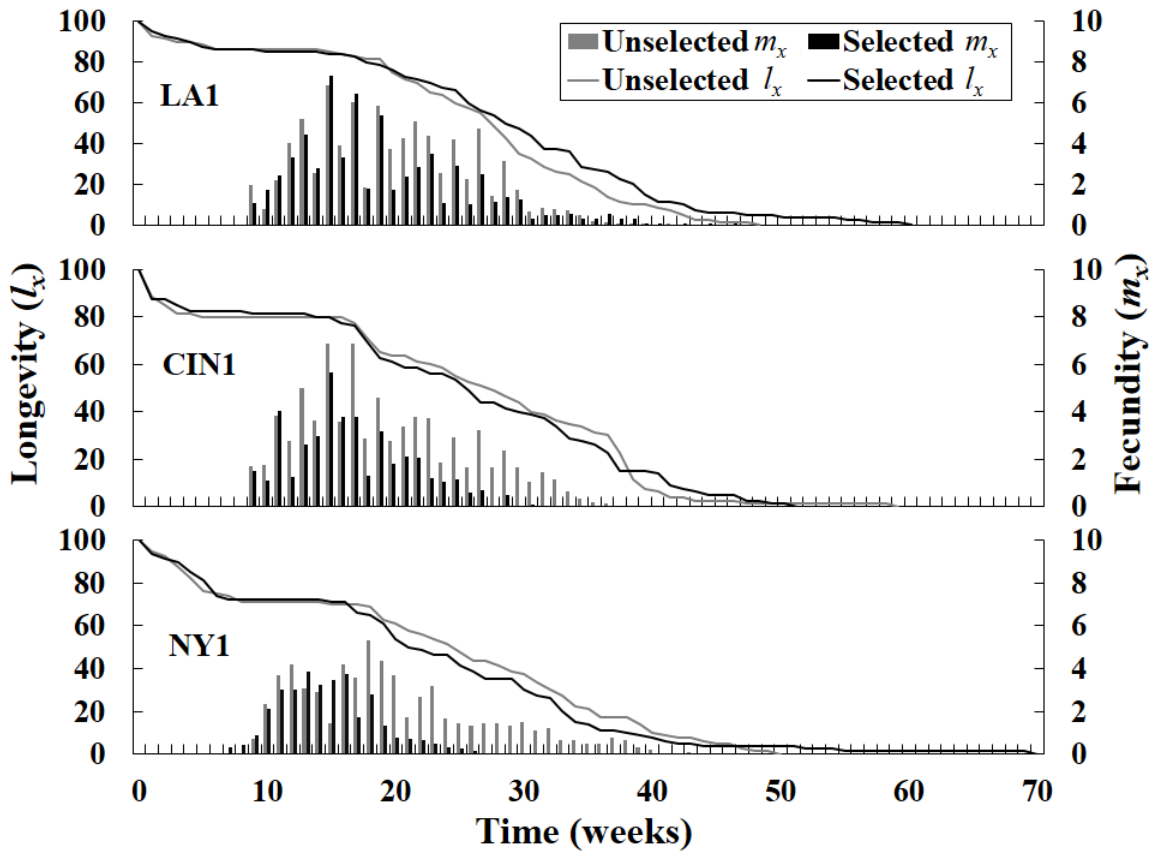
Survival (l_x; left axis and curve) and oviposition rate (m_x; right axis and histogram) over time for unselected and selected strains. Grey represents the unselected strains, and black represents the selected strains. Adult survival reached 50% at 31.1 (± 0.94), 31.6 (± 2.60) and 34.0 (± 3.30) weeks for the unselected LA1, CIN1 and NY1 strains, respectively, and at 32.5 (± 1.58), 30.6 (± 2.34) and 28.6 (± 2.77) weeks for the paired selected strains. The average time from the egg to adult molt was 10.8 (± 0.63), 10.0 (± 0.70), 10.3 (± 0.25) weeks for the unselected LA1, CIN1 and NY1 strains, respectively, and 10.5 (± 0.87), 9.3 (± 0.63), 10.3 (± 0.85) weeks for the paired selected strains (Figures 4.3, 4.4 and 4.5). Once oviposition began for a cohort of bugs, it was sustained for 11-34 weeks.

Figure 4.4
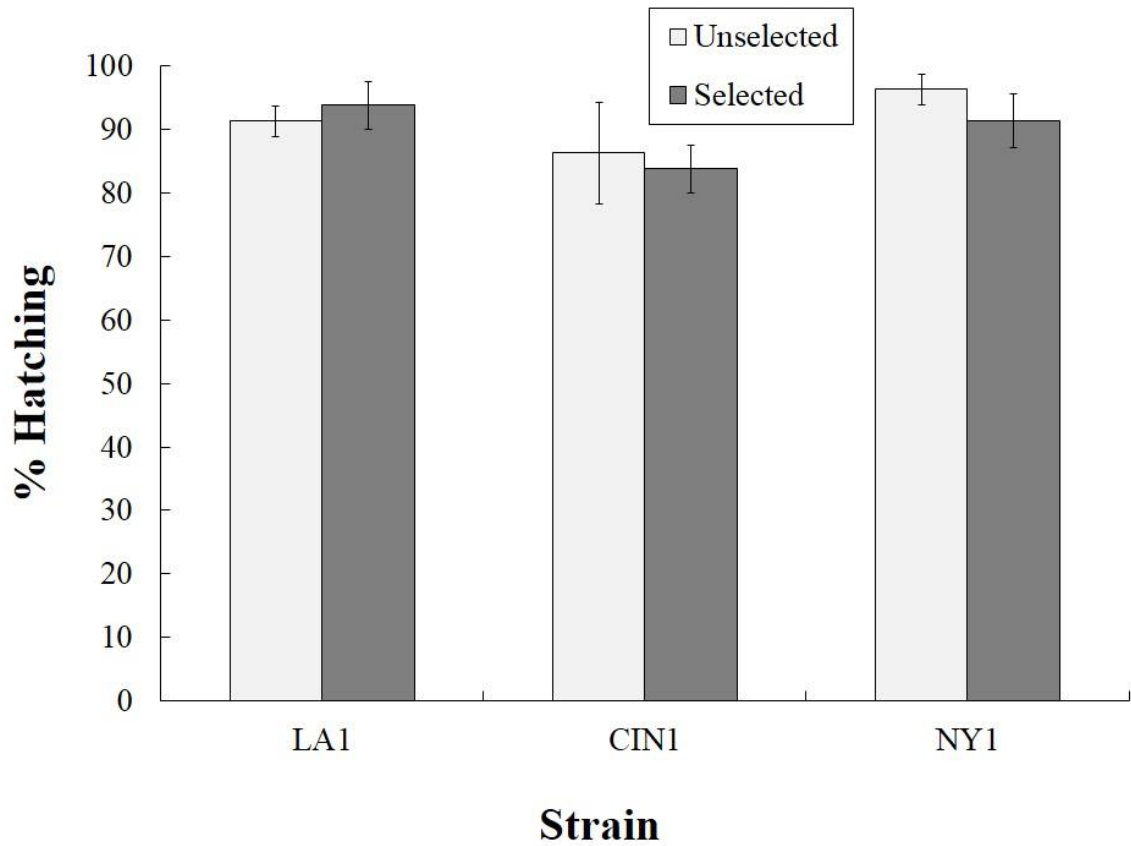
Source of variation	Sum of Squares	d.f.	Mean square	F-stat	Significance
Treatment	16.67	1	16.67	0.23	0.636
Strain	358.33	2	179.17	2.50	0.112
Replicate(strain)	300.00	3	100.00	1.39	0.279

Mean per cent hatch rate of eggs laid by F_1 mothers. For unselected LA1, CIN1 and NY1, the mean was 91.3 ($\pm$ 2.4), 86.3 ($\pm$ 8.0) and 96.3 ($\pm$ 2.4), respectively, and for selected it was 93.8 ($\pm$ 3.8), 83.8 ($\pm$ 3.8) and 91.3 ($\pm$ 4.3), respectively. The table includes statistics describing the effects of treatment, strain and replicate within strain.

Figure 4.5
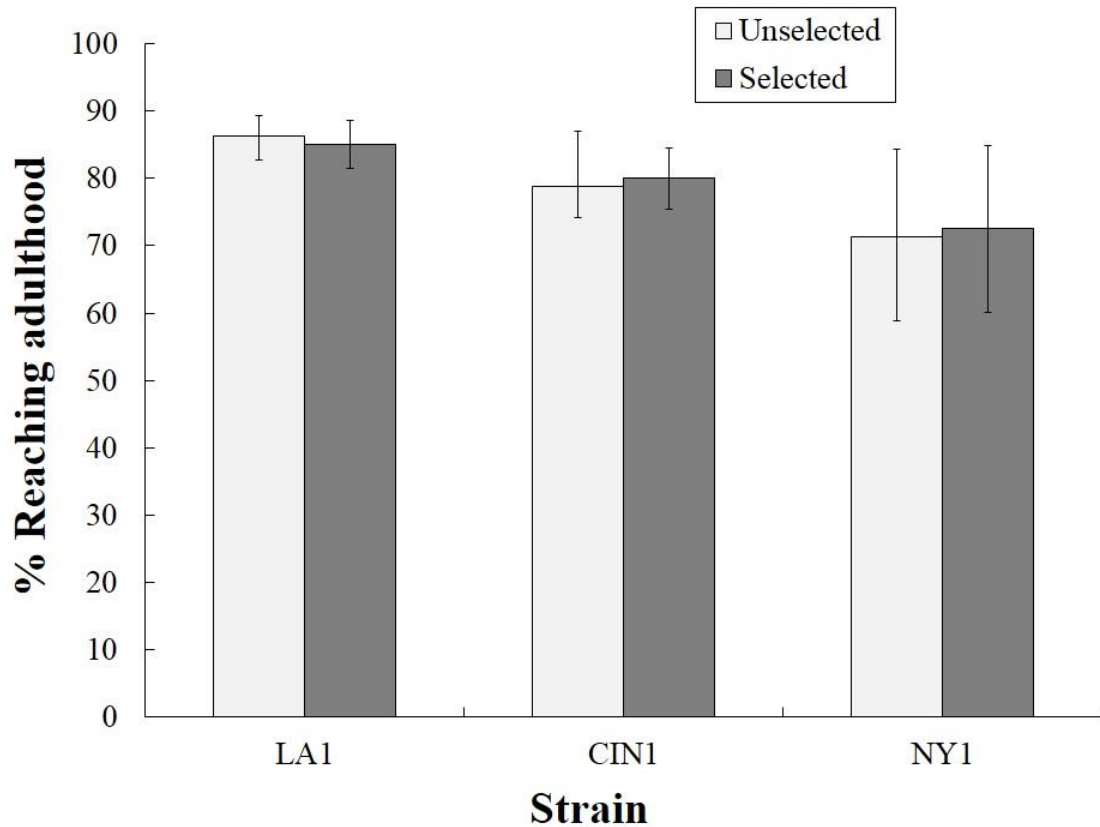
Source of variation	Sum of Squares	d.f.	Mean square	F-stat	Significance
Treatment	1.04	1	1.04	0.01	0.925
Strain	758.33	2	379.17	3.30	0.062
Replicate(strain)	3,284.38	3	1,094.79	9.52	0.001

Mean per cent of eggs that reached adulthood. For unselected LA1, CIN1 and NY1, the mean was 86.3 (± 3.1), 78.8 (± 8.3) and 71.3 (± 13.1), respectively, and for selected it was 85.0 (± 3.5), 80.0 (± 4.6) and 72.5 (±12.3), respectively. The table includes statistics describing the effects of treatment, strain and replicate within strain.

Figure 4.6
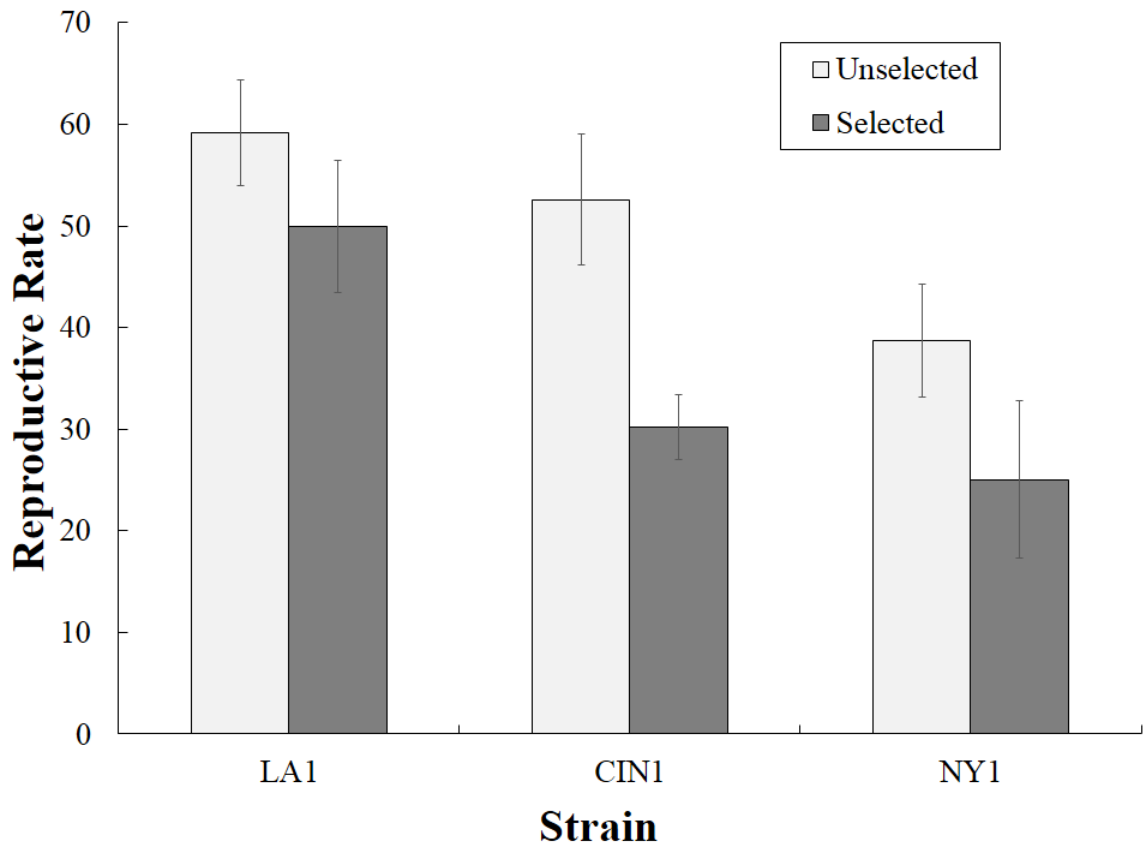
Source of variation	Sum of Squares	d.f.	Mean square	F-stat	Significance
Treatment	1,362.45	1	1,362.45	12.17	0.003
Strain	2,078.11	2	1,039.05	9.28	0.002
Replicate(strain)	821.55	3	273.85	2.45	0.099

Mean reproductive rate. For unselected LA1, CIN1 and NY1, the mean was 59.2 (± 5.2), 52.6 (± 6.4) and 38.7 (± 5.6), respectively, and for selected it was 50.0 (± 6.5), 30.2 (± 3.2) and 25.0 (± 7.7), respectively. The table includes statistics describing the effects of treatment, strain and replicate within strain.

Figure 4.7
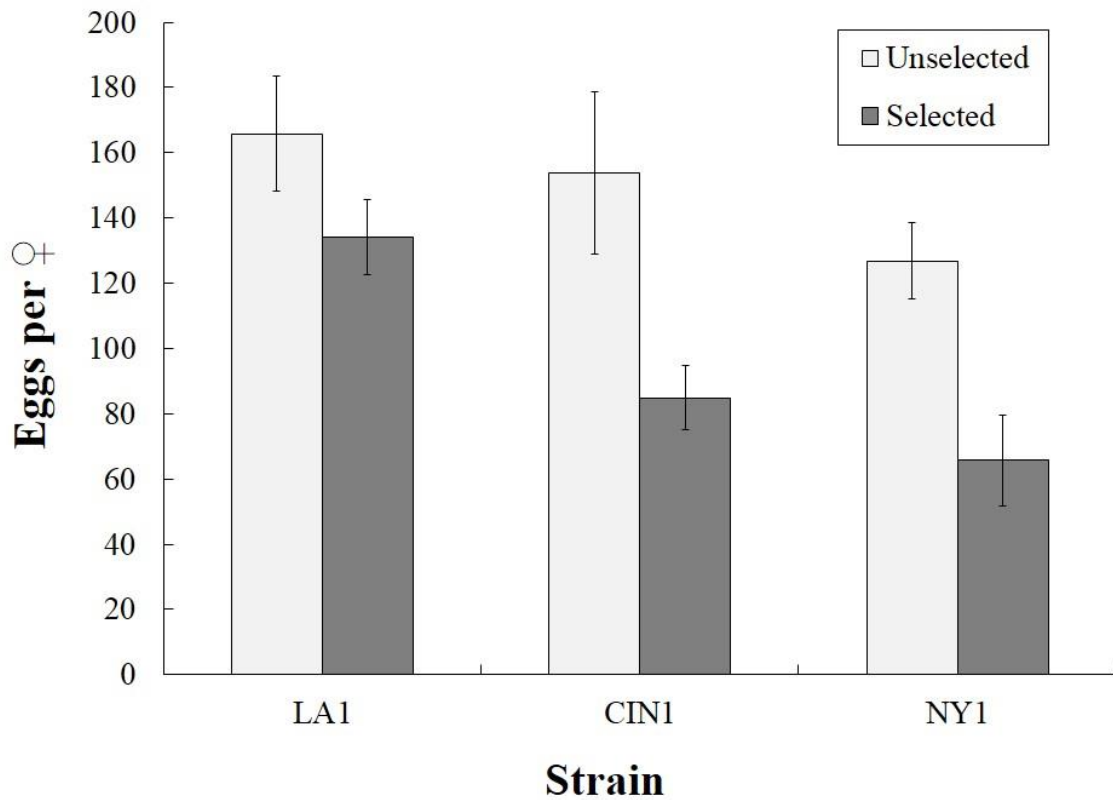
Source of variation	Sum of Squares	d.f.	Mean square	F-stat	Significance
Treatment	17,463.62	1	17,463.62	15.41	0.001
Strain	11,634.39	2	5,817.20	5.13	0.018
Replicate(strain)	138.10	3	46.03	0.041	0.989

Average number of eggs per female. For unselected LA1, CIN1 and NY1, the mean was 165.8 ($\pm$ 17.7), 154.0 ($\pm$ 24.9) and 126.8 ($\pm$ 11.6), respectively, and for selected it was 134.2 ($\pm$ 11.5), 84.9 ($\pm$ 9.7) and 65.7 ($\pm$ 13.9), respectively. The table includes statistics describing the effects of treatment, strain and replicate within strain.

Figure 4.8
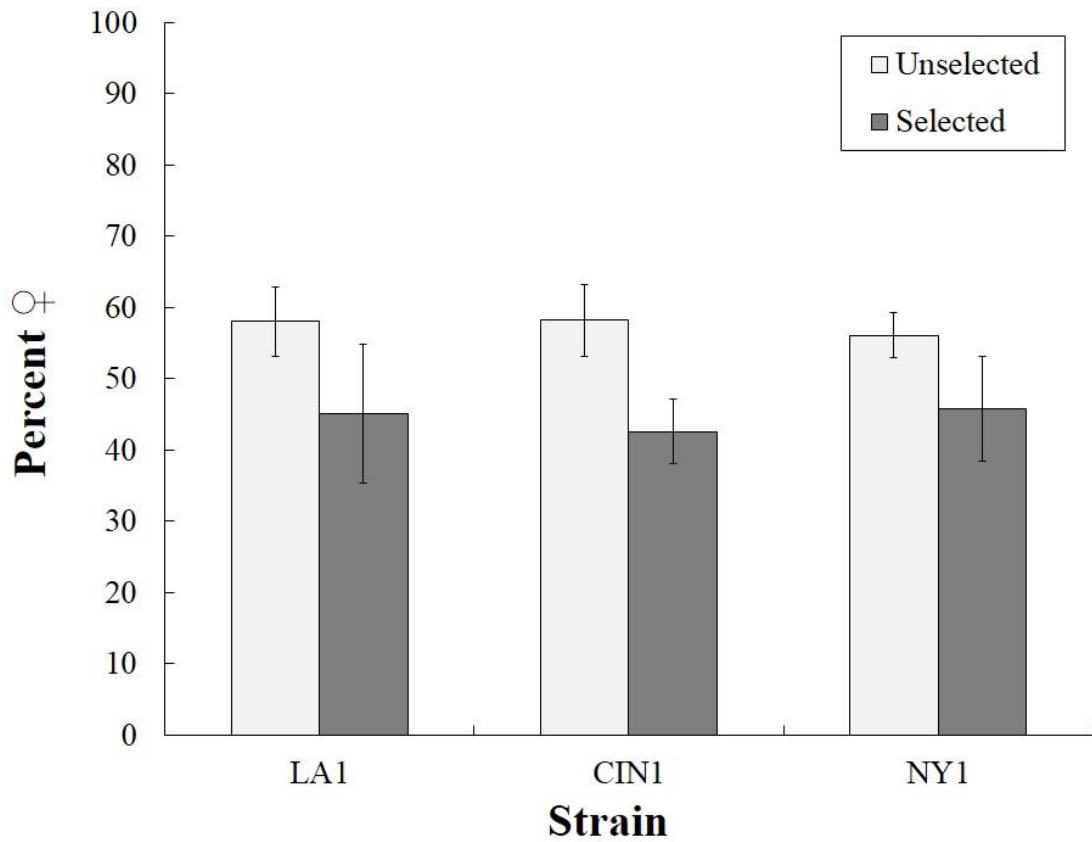
Source of variation	Sum of Squares	d.f.	Mean square	F-stat	Significance
Treatment	0.10	1	0.10	7.00	0.017
Strain	0.00	2	0.00	0.01	0.982
Replicate(strain)	0.03	3	0.01	0.74	0.542

Proportion of adults that are female. For unselected LA1, CIN1 and NY1, the mean was 58.0 ($\pm$ 4.9), 58.2 ($\pm$ 5.0) and 56.1 ($\pm$ 3.2), respectively, and for selected it was 45.1 ($\pm$ 9.7), 42.6 ($\pm$ 4.6) and 45.8 ($\pm$ 7.3), respectively. The table includes statistics describing the effects of treatment, strain and replicate within strain.

Figure 4.9
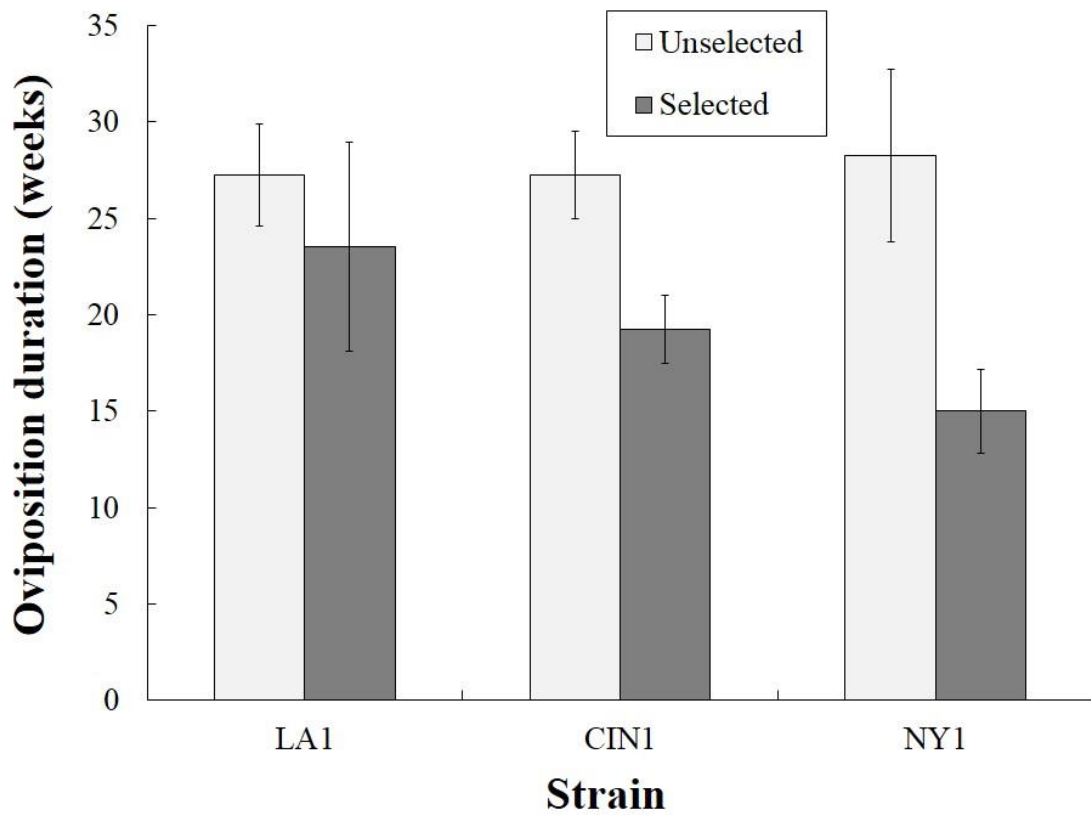
Source of variation	Sum of Squares	d.f.	Mean square	F-stat	Significance
Treatment	416.67	1	416.67	9.08	0.008
Strain	56.583	2	28.29	2.86	0.085
Replicate(strain)	20.28	3	6.76	1.39	0.279

Mean oviposition duration in week. For unselected LA1, CIN1 and NY1, the mean was 27.3 (± 2.7), 27.3 (± 2.3) and 28.3 (± 4.5), respectively, and for selected it was 23.5 (± 5.4), 19.3 (± 1.8) and 15.0 (± 2.2), respectively. Oviposition duration was calculated by halving the difference of the last week of oviposition by the first week. The table includes statistics describing the effects of treatment, strain and replicate within strain.

Chapter 5. Variation in susceptibility to commonly used insecticides among populations of bed bugs: Is there an opportunity for resistance management?

Introduction

The recent global resurgence in populations of the bed bug, *Cimex lectularius*, has proven to be a difficult challenge for pest management professionals (Potter et al. 2010, 2011 and 2013). Bed bugs were nearly eliminated in North America, Europe and elsewhere after the discovery and widespread use of DDT and other chlorinated insecticides in the 1940s (Doggett et al. 2008, Kilpinen et al. 2011, Mumcuoglu and Shalom 2010, Omudu and Kuse 2010, Bencheton et al. 2011 and Tawatsin et al. 2011). The bugs' resurgence is likely due to several factors, including the evolution of resistance to insecticides that target sodium ion channels, including pyrethroids and DDT (Romero et al. 2007, Steelman et al. 2008, Yoon et al 2008, Mamidala et al. 2011 and Zhu et al. 2010). Pyrethroid resistance has created a demand for insecticides with different modes of action, as well as alternative control tactics such as heat, mattress encasements, steam, and different classes of insecticides (Wang et al. 2009a, Romero et al. 2010, Potter et al. 2011 & 2012, Gordon et al. 2014a).

Integrated pest management (IPM) is an approach that has been used to successfully control many different pest species safely and effectively by utilizing multiple control tactics. For many pest systems, a successful IPM strategy will utilize mechanical, cultural and chemical control methods to manage the pest and additional approaches to monitor or detect pest numbers (Croft 1990, Bennett et al. 2003, Onstad 2008). For bed bugs, this means using visual inspections, traps and canines for detection and mattress encasements, heat, steam, vacuums and other methods in conjunction with

insecticides (e.g., pyrethroids, neonicotinoids, pyrroles, desiccant dusts and essential oils) for control (Pfiester et al. 2008, Wang et al. 2009b & 2011, Potter et al 2011, Gordon et al. 2014, Potter et al. 2014). In addition to multiple methods, a sustainable IPM program will monitor insecticide resistance and make insecticide recommendations based on the results (Bennett et al. 2003). For bed bugs, no such resistance monitoring device exists, nor does it exist for other urban pests such as cockroaches. However, one research group recently showed the validity of this idea (Dang et al. 2014a), and our group is working with a commercial entity to bring such a diagnostic tool to market. Although many pest management companies utilize IPM strategies, the industry as a whole still relies heavily on insecticides (Potter and Haynes 2014).

Currently, the industry standard for bed bug control is the use of residual insecticides containing a pyrethroid component. In a recent survey conducted by the National Pest Management Association, eight out of the top 10 products used for bed bug control by pest management professionals contained a pyrethroid (Potter et al. 2013). However, some of these products are more effective than others (Moore and Miller 2006, Romero et al. 2007, Gordon et al. 2014a).

In the current study, we investigated the effectiveness of nine commercial insecticides on six populations of bed bugs. Based on earlier work, we expected that susceptibility would vary among populations based on the history of insecticide exposure. Insecticide bioassays were used to investigate this hypothesis.

 Materials and Methods

Insects. Six populations of bed bugs were used in this study (Table 5.1). Insects were housed in incubators at 26.7° C, 65 ± 5% RH, and a photoperiod of 14:10 (L:D) h.

All bed bugs were fed weekly on defibrinated rabbit blood (Quad Five, Ryegate, MT) using the methods of Montes et al. (2002).

  Product Evaluations. A residual bioassay (Romero et al. 2007) was used to determine susceptibility of six different populations of bed bugs to nine different commercial insecticides: Temprid SC (β-cyfluthrin/imidacloprid; Bayer; Research Triangle Park, NC), Suspend SC (deltamethrin; Bayer; Research Triangle Park, NC), Tempo SC (β-cyfluthrin; Bayer; Research Triangle Park, NC), Transport GHP and Mikron (bifenthrin/acetamiprid; FMC; Philadelphia, PA), Tandem SC (λ-cyhalothrin/thiamethoxam Syngenta; Greensboro, NC), Alpine WSG (dinotefuran; BASF; Research Triangle Park, NC), Phantom SC (chlorfenapyr; BASF; Research Triangle Park, NC) and CimeXa (silica gel; Rockwell Labs; North Kansas City, MO). Individual wells of a 24-well cell culture plate (Costar, Corning, NY) were lined with filter paper disks (Whatman #2, cut to 1.7 cm diam.). Label rate solutions (Table 5.2) were made by diluting the concentrated insecticides in water. Fifty μL of each solution was pipetted onto the filter papers fitted into the wells, and then allowed to dry completely before bugs were placed on the surface. Mortality was scored after 4 h, 1, 2, 4, 8 and 14 d continuous exposure to the treated filter papers. Insects were classified as dead (including moribund) if they showed no movement or were unable to right themselves within 15 s of being inverted with soft forceps.

  Data Analysis. Biostat 2009 was used to perform probit analysis that calculated the lethal time that kills 50 % of the population (LT_{50}; AnalystSoft Inc. 2009). Abbott's (1925) formula was used to correct for control mortality for probit analysis. Minitab was also used to perform a multiple comparison test for proportions to compare differences in

mortality among populations (Zar 1999). Correlation analysis was performed using Statistix 10.0 (Analytical Software 2013) to investigate the relationship between different insecticidal product efficacies.

Results and Discussion

   Product evaluations. Insecticidal products can be divided somewhat arbitrarily into two groups based on speed of action: fast-acting or slow-acting. Similarly, the relative effectiveness at killing entire populations can be divided into three categories: ultimately effective, moderately effective or ineffective. CimeXa, a desiccant dust, was the only insecticide that was uniformly both fast-acting and ultimately effective. Phantom SC was slow-acting but ultimately effective. The rate of effectiveness to Transport GHP ranged from slow-acting to fast-acting but was ultimately effective with variation among populations in shape of the mortality curves. Suspend SC, Tempo SC, Temprid SC and Tandem SC were fast-acting when exposure resulted in any mortality. Transport Mikron was slow-acting, and the efficacy ranged from ultimately effective to ineffective. Alpine WSG was the only insecticide that was uniformly ineffective (Figure 5.1).

   Suspend SC. The LT_{50}s for Suspend SC ranged from <4 hours to >336 hours (Table 5.3); however, an accurate LT_{50} could not be calculated for CIN10, FF1 or LEX6 due to low mortality after 336 continuous hours of exposure (Figure 1 A). A multiple comparisons test placed the six populations into three distinct groups [here we call them susceptible (LA1 and CO1), moderately resistant (LEX8) and resistant (CIN10, FF1 and LEX6)] after 24 hours of exposure (Table 5.4). By 96 hours of exposure, the sequence of susceptibilities of the populations was similar (Table 5.5).

   Tempo SC. An accurate LT_{50} could only be calculated for one population due to

79

extremely high initial mortality after 4 hours or no mortality after 336 h (Table 5.3). The

Tukey-like test for comparison of multiple proportions created the same three groups of

susceptibility delineated from susceptibility to Suspend SC (CO1 = LA1 > LEX8 >

CIN10 = FF1 = LEX6; Tables 5.4 and 5.5). Tempo SC has the same pyrethroid

component as Temprid SC (ß-cyfluthrin); however, Tempo SC was more effective than

Temprid SC against LA1 despite Temprid SC containing an additional active ingredient

(neonicotinoid; Figure 5.1 B).

Alpine WSG. Susceptibility of all strains to Alpine WSG was unique compared to

all other products investigated (Figure 5.1 C). An LT_{50} could not be calculated for any

population due to insufficient mortality after 336 h of exposure (Table 5.3). Populations

did not vary in susceptibility to Alpine WSG after 24 h of exposure (Table 5.4).

Mortality at 336 h was never greater than 41.7 %.

Temprid SC. Susceptibility to Temprid SC closely resembled that of Tempo SC,

Suspend SC and Tandem SC (Figure 5.1 D). An accurate LT_{50} could only be calculated

for LEX8 (Table 5.3). The Tukey-like test for comparison of multiple proportions

grouped populations into three categories of susceptibility that did not vary between 24 h

and 96 h of exposure (CO1 = LA1 > LEX8 > CIN10 = FF1 = LEX6; Tables 5.4 and 5.5).

Interestingly, Temprid SC was only one of three commercial insecticides that did not

cause 100% mortality of LA1 after 336 h of continuous exposure.

Tandem SC. As for all products containing a pyrethroid (with the exception of

Transport GHP), LT_{50}s could not be calculated for the majority of populations due the

rate of efficacy being either faster than 24 h or slower than 14 d (Table 5.3). Tandem SC

was initially fast acting against LEX8 (4 h = 43.3%) but only moderately effective

(Figure 5.1 E). In contrast, Tandem SC was slow-acting but ultimately effective against CO1. Statistical analyses initially divided populations into two groups after 24 h of exposure (Table 5.4); however, after 96 h of exposure, three groups emerged that followed the pattern observed for Suspend SC, Temprid SC and Tempo SC (CO1 = LA1 > LEX8 > CIN10 = FF1 = LEX6; Table 5.5).

Transport GHP. Transport GHP was one of only three insecticides that was ultimately effective for all populations (Figure 5.1 F). The LT_{50}s of populations for Transport GHP ranged from <4 h to 99.2 h (Table 5.3). Analysis of mortality after 24 h show two groups of susceptibility (Table 5.4), but after 96 h, populations could be divided into three groups of susceptibility with LEX6 and LEX8 being intermediate between the two extreme groups (Table 5.5).

Transport Mikron. Transport Mikron was the least effective product out of all insecticides containing a pyrethroid investigated (Figure 5.1 G), and exposure to residual deposits was ultimately effective for only one population (CO1). The LT_{50}s for this insecticide ranged from <4 h to >336 h and could only be accurately calculated for CO1 and LEX8 (Table 5.3). Even though Transport Mikron was initially fast-acting against LA1, it was only moderately effective (Figure 5.1 G). The Tukey-like test for comparison of multiple proportions divided populations into two distinct groups. Initially, CO1 was intermediate between the extremes (Table 5.4), but after 96 h of exposure, CO1 fell solely into the most susceptible group (Table 5.5). Because Transport Mikron and Transport GHP share the same active ingredients at the same label rate, the consistently elevated mortality stimulated by GHP suggests that bioavailability is enhanced in this product, possibly due to differences in formulation (Gordon et al.

2014b). At 96 h (Table 5.5), mortalities were 20.7 to 67.9% higher with GHP among the populations tested.

Phantom SC. Phantom SC was slow-acting and ultimately effective; however, one population still had 4.8% survival after 336 h of continuous exposure (Figure 5.1 H). The LT_{50} values ranged from 48.3- 172.5 h (Table 5.3).   The LEX8 strain of bed bugs achieved 100.0 % mortality after 8 days of exposure; whereas the pyrethroid-susceptible strain of bugs (CO1) had the lowest mortality of all populations after the same duration of exposure (8 days = 45.5 %).   There were differences among populations at 24 and 96 h that disappeared after 336 h. (Tables 5.4 and 5.5).

CimeXa. CimeXa was fast-acting and ultimately effective for all populations investigated (Figure 5.1 I). The LT_{50} values for all populations fell within the range of 4-24 h (Table 5.3). All populations fell into one category based on susceptibility (Tables 5.4) and did not change over time (Table 5.5).

Correlation analysis. A correlation analysis clarifies the similarity and contrasts of mortalities among insecticides, and may provide guidance for the selection of alternative insecticides when resistance to one is suspected (Table 5.6). High correlation values indicate that the insecticide pair stimulated similar levels of mortality within populations. For example, the high correlation coefficient found between Suspend SC and Tempo SC (r = 0.99) occurred, because both products are effective against the same populations (CO1, LA1), moderately effective against LEX8 and less effective against a different set of populations (CIN10, FF1, LEX6). If Suspend SC was ineffective against a certain population, it would be most reasonable to switch to an insecticide that is weakly or negatively correlated with Suspend SC (e.g., Phantom SC or CimeXa; CimeXa is not

shown in Table 5.6, because the insecticide has no correlation with any insecticide due to its uniform effectiveness against populations). Before a pest management technician would choose an insecticide for an account, one would need to rely on information from the correlation matrix and information from the 96 h (Table 5.5) mortality table to make a choice for an alternate insecticide, because low correlations can result from an insecticide being uniformly ineffective.

The high correlations between pyrethroid (Suspend SC and Tempo SC) and neonicotinoid/pyrethroid combinations (Temprid, Tandem, Transport) suggest that the addition of the neonicotinoid does not lead to independence of action from the pyrethroid-only products. This supposition is further strengthened by the fact that Alpine WSG, the only single action, neonicotinoid based commercial insecticide available for bed bug control, was consistently ineffective.

General discussion. Over the last 15 years, there has been a global resurgence in populations of *C. lectularius* (Doggett et al. 2008, Potter et al. 2010, Kilpinen et al. 2011, Mumcuoglu and Shalom 2010, Omudu and Kuse 2010, Bencheton et al. 2011 and Tawatsin et al. 2011) and these new populations are challenging pest management firms by being difficult to control. In two recent surveys conducted by the National Pest Management Association asking, "What is the most difficult insect to control?" pest managers overwhelming responded "bed bugs" each time (Potter et al. 2011 and 2013). Factors contributing to the difficulty with control are numerous and include insecticide resistance, difficulties in locating pest harborage sites due to excess clutter and the bug's cryptic nature, and the unique attributes of bed bug dispersal and establishment (passive dispersal resulting in few establishing members).

Most bed bug infestations in the field are genetically unique due to the extreme genetic bottle neck populations must go through before establishing (Booth et al. 2012). One study found that the majority of infestations are started with just one or two founding females mated by males closely related to her (Booth et al. 2012). Due to the relatively few establishing members and limited genetics, each successful population of bed bugs has extreme founder effects. Similar studies have shown that the gene flow between populations is also limited as characterized by high genetic diversity between populations (Booth et al. 2012, Saenz et al. 2012, and Fountain et al. 2014). As a result, any population of bed bugs encountered by a pest control operator (PCO) may require a unique control strategy tailored specifically to the individual infestation (i.e., the use of specific insecticides, alone or in conjunction with non-insecticidal management practices such as mattress encasements, steaming, heat, etc.).

Results from this study confirm that a single population of bed bugs can react differently to commercial insecticidal products containing similar active ingredients. Some of the observed differences in susceptibility could be attributed to formulation. Each commercial product will contain a unique blend of inert ingredients, synergists and active ingredients that may result in varied bioavailability to the bug. Recently, one study found that even though Transport Mikron and GHP contain the same ratio and concentration of active ingredients, the difference in formulation may affect the effectiveness (Gordon et al. 2014b). In addition to formulation, the physiology of the bed bug (i.e., up-regulation of enzymes, altered target sites and changes in the cuticle) may alter the effectiveness of an insecticide (Romero et al. 2007, Steelman et al. 2008, Yoon et al 2008, Mamidala et al. 2011 and Zhu et al. 2010). Considering that populations of *C.*

lectularius have differing susceptibilities to insecticides, pest managers would benefit from knowing which products perform best against each population encountered.

An approach to prescreen bed bugs using various insecticides from an infested dwelling would enable a PCO to prescribe the most appropriate treatment for the specific population. Prior to treatment, pest control personnel typically inspect the premises looking for signs and locations of bed bugs. If the practitioner had a resistance monitoring 'kit' at this time, he could collect a sample of bed bugs from the infested dwelling and place them on insecticide pretreated surfaces prior to performing treatment. Bed bug treatments often need to be scheduled following the initial inspection, allowing the PMP enough time to assay bugs and make decisions based on predicted mortalities with candidate insecticides (Table 5.3). Utilizing this approach would help practitioners choose the most effective and efficient insecticides and eliminate the infestation as quickly as possible using lesser amounts of material.

Monitoring insecticide resistance is the first step in an effective insecticide resistance management plan for controlling populations of bed bugs. The use of such a device would be unique for urban pest management; however, this idea is not unique for other pest systems. The World Health Organization maintains a website, supplies pretreated bottles and gives recommendations about how to evaluate insecticide resistance in mosquitoes in order to make educated recommendations for abatement (Brogdon and McAllister 1998, Perea et al. 2009). Utilizing a susceptibility monitoring system refined for use with bed bugs would facilitate selection of products and practices and aid in effective and responsible control of this pest.

Table 5.1. Origins of bed bug populations

that were evaluated for their

susceptibility to commercial insecticide

products

Strain	Origin[a]	Year[b]
CIN10	Cincinnati, OH	2012
CO1[c]	Collinsville, MS	2013
FF1	Frankfort, KY	2012
LA1	Los Angeles, CA	2007
LEX6	Lexington, KY	2012
LEX8	Lexington, KY	2012

[a] City from which the collection originated.

[b] The year the strain began culture in the lab.

[c] This population was collected from a chicken coop. All others populations were from human dwellings.

Table 5.2. Commercial insecticides used in this study

Product[a]	Class[b]	Active ingredient(s)	% A.I.[c]
Suspend SC	Pyrethroid	deltamethrin	0.06
Tempo SC	Pyrethroid	β-cyfluthrin	0.05
Alpine WSG	Neonicotinoid	dinotefuran	0.3
Temprid SC	Pyrethroid/Neonicotinoid	β-cyfluthrin/imidacloprid	0.025/0.05
Tandem SC	Pyrethroid/Neonicotinoid	λ-cyhalothrin/thiamethoxam	0.03/0.10
Transport GHP	Pyrethroid/Neonicotinoid	bifenthrin/acetamiprid	0.06/0.05
Transport Mikron	Pyrethroid/Neonicotinoid	bifenthrin/acetamiprid	0.06/0.05
Phantom	Pyrrole	chlorfenapyr	0.5
CimeXa	Desiccant dust	silica gel	100

[a] Trade name of insecticide registered for bed bug control.

[b] The insecticidal class(es) of active ingredients in commercial product

[c] The per cent of active ingredient of label rate material.

Table 5.3. Lethal time to kill 50 % of each population of bed bugs for nine commercial insecticides

Strain	Suspend	Tempo	Alpine	Temprid	Tandem	Transport GHP	Transport Mikron	Phantom	CimeXa
CIN10	> 336[a]	> 336	> 336	> 336	> 336	49.2 (0.0-123.2)	> 336	57.4 (34.0-88.3)	4-24
CO1	6.4 (5.4-7.6)	< 4	> 336	< 4	14.0 (11.7-16.4)	< 4	50.3 (35.9-66.7)	172.5 (106.9-273.4)	4-24
FF1	> 336	> 336	> 336	> 336	> 336	99.2 (67.4-136.1)	> 336	156.5 (47.5-515.8)	4-24
LA1	< 4	< 4	> 336	< 4	< 4	< 4	< 4	120.3 (110.6-130.7)	4-24
LEX6	> 336	> 336	> 336	> 336	> 336	61.2 (33.5-84.1)	> 336	168.9 (157.5-180.7)	4-24
LEX8	23.2 (15.9-31.5)	36.4 (23.0-53.8)	> 336	42.0 (23.0-70.5)	11.3 (5.8-17.9)	15.0 (7.6-23.9)	151.3 (72.5-642.4)	48.3 (44.3-52.5)	4-24

[a] LT_{50} values were calculated using Biostat 2009 (AnalystSoft, Inc. 2009) and could not be calculated for populations with mortality greater than 90% after 4 h (denoted as <4) or less than 10% mortality after 336 h (denoted as >336).

Table 5.4. Mortality after 24 hours of exposure to residual deposits of nine commercial insecticides commonly used for bed bug control

Strain	Suspend[a]	Tempo	Alpine	Temprid	Tandem	Transport GHP	Transport Mikron	Phantom	CimeXa
CIN10	3.3c	0.0c	0.0a	0.0c	0.0b	n/a	3.3b	20.0a	96.7a
CO1	96.7a	100.0a	3.3a	100.0a	70.0a	100.0a	23.3ab	0.0b	n/a
FF1	0.0c	0.0c	0.0a	0.0c	n/a[b]	n/a	n/a	3.3ab	96.6a
LA1	86.7a	96.7a	0.0a	83.3a	n/a	n/a	n/a	0.0b	100.0a
LEX6	3.3c	0.0c	0.0a	0.0c	0.0b	n/a	n/a	0.0b	100.0a
LEX8	50.0b	46.7b	0.0a	41.7b	60.0a	53.3b	36.7a	13.3ab	96.7a

[a] Control mortality never exceeded 5.0 %, thus, Abbott's (1925) formula was never used. A Tukey-type multiple comparison of proportions test was used to investigate if populations varied significantly in susceptibility to an insecticide. Different letters within a column denotes a significant difference ($p \leq 0.05$).

[b] Mortality was not taken for some populations at 24 h.

Table 5.5. Mortality after 96 hours of exposure to residual deposits of nine commercial insecticides commonly used for bed bug control

Strain	Suspend[a]	Tempo	Alpine[b]	Temprid	Tandem	Transport GHP	Transport Mikron	Phantom	CimeXa
CIN10	10.0*c*	3.3*c*	0.0a*b*	0.0*c*	0.0*c*	53.3*c*	6.7*b*	56.7*b*	100.0*a*
CO1	100.0*a*	100.0*a*	11.7*a*	100.0*a*	100.0*a*	100.0*a*	80.0*a*	16.7*c*	n/a
FF1	0.0*c*	0.0*c*	1.7a*b*	13.3*c*	0.0*c*	40.0*c*	0.0*b*	10.0*c*	100.0*a*
LA1	90.0*ab*	100.0*a*	0.0*b*	93.3*a*	100.0*a*	100.0*a*	80.0*a*	33.3*bc*	100.0*a*
LEX6	3.3*c*	0.0*c*	0.0a*b*	3.3*c*	0.0*c*	70.0*bc*	0.0*b*	6.7*c*	100.0*a*
LEX8	73.3*b*	63.3*b*	13.3*a*	55.0*b*	76.6*b*	93.3*ab*	46.7*a*	96.7*a*	100.0*a*

[a] Control mortality never exceeded 18.5%, thus, Abbott's (1925) formula was never used. A Tukey-type multiple comparison of proportions test was used to investigate if populations varied significantly in susceptibility to an insecticide. Different letters within a column denotes a significant difference ($p \leq 0.05$).

[b] CIN10 and LEX6 had a sample size of 30, whereas all other populations had a sample size of 60 when evaluating Alpine. A Tukey-type multiple comparison test factors in sample size to calculate significance; thus, differences in susceptibility between populations may be affected by the limited samples sizes relative to other populations.

Table 5.6. Correlation coefficients of mortality at 96 hours for eight commercial insecticidal products

Insecticide	Suspend	Tempo	Alpine	Temprid	Tandem	Transport GHP	Transport Mikron	Phantom
Suspend	1.0000[a]							
Tempo	0.9913	1.0000						
Alpine	0.6244	0.5319	1.0000					
Temprid	0.9718	0.9898	0.5231	1.0000				
Tandem	0.9953	0.9939	0.5949	0.9784	1.0000			
Transport GHP	0.9292	0.9143	0.5544	0.8722	0.9270	1.0000		
Transport Mikron	0.9863	0.9982	0.5004	0.9877	0.9861	0.9034	1.0000	
Phantom	0.2724	0.1791	0.4781	0.0680	0.2550	0.2618	0.1594	1.0000

[a] Correlation coefficients of mortality at 96 h from six populations of bed bugs (CIN10, CO1, FF1, LA1, LEX6 and LEX8) calculated using Minitab (Zar 1999). CimeXa has no correlation with any insecticide due to its uniform effectiveness against all populations.

Figure 5.1
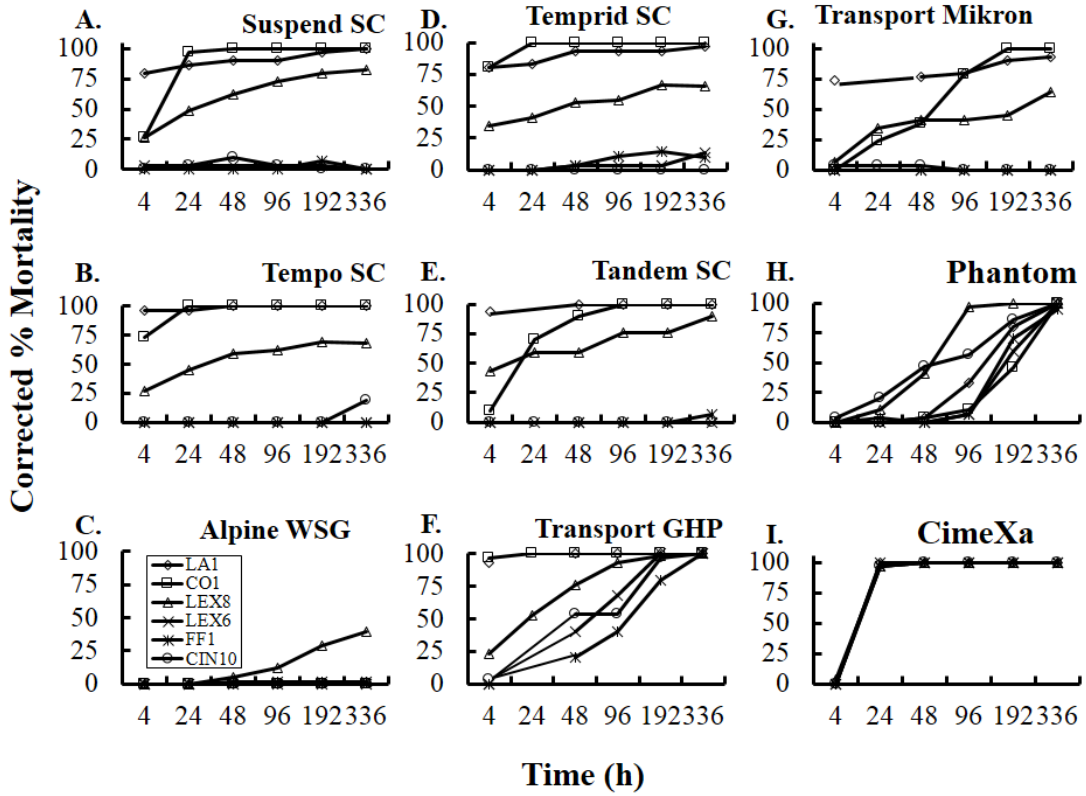
Mortality over time of six populations of bed bugs to nine commercial products approved for bed bug control: graph A represents Suspend SC, graph B represents Tempo SC, graph C represents Alpine WSG, graph D represents Temprid SC, graph E represents Tandem SC, graph F represents Transport GHP, graph G represents Transport Mikron, graph H represents Phantom SC and graph I represents CimeXa.

Chapter 6. Conclusion and future directions

Insecticide resistance is a worldwide phenomenon and has been documented in hundreds of insect species to most, if not all, classes of insecticides (Forgash 1984, Georghiou 1986). The discovery and development of new classes of insecticides is limited, especially for use in the urban environment (Potter and Haynes 2014). Given this information, individuals tasked with controlling pest populations must manage resistance to avoid losing entire classes of insecticides. Insecticide resistance management (IRM) strategies ultimately allow professionals to eliminate resistant pest populations and preserve current classes of insecticides. An IRM program is just one component of a successful integrated pest management program. Monitoring insecticide resistance could allow one to choose the most effective product that continues to control the insect population while relieving selection pressure for resistance (Croft 1990, Bennett 2003, Onstad 2008). Insecticide resistance is one factor that has been implicated in the recent global resurgence in populations of bed bugs, *Cimex lectularius* (Romero et al. 2007, Steelman et al. 2008, Yoon et al. 2008, Zhu et al. 2010, Mamidala et al. 2011). In the past, populations of bed bugs were effectively and efficiently controlled with DDT and other broad-spectrum, residual insecticides (Potter 2011). However, resistance to pyrethroid insecticides has created challenges for pest management professionals (PMP; Potter et al. 2010, 2011 and 2013). The purpose of this dissertation was to document the evolution of resistance to pyrethroid and neonicotinoid combination products and to a neonicotinoid in the laboratory, to document potential fitness costs to resistance to the combination products, and to compare the efficacy of nine insecticides on six populations.

Dual action combination products that contain both a pyrethroid and neonicotinoid are currently some of the most effective and widely used insecticides by PMPs due to their long residual activity (Potter et al. 2012). These commercial products stack two different modes of action with the goal of better controlling populations than either class of insecticides could alone; however, given that resistance to one of these classes (pyrethroids) has already been extensively documented (Romero et al. 2007, Steelman et al. 2008, Yoon et al. 2008, Mamidala et al. 2011) evolution of resistance to the combination products is of great concern. The first issue investigated in this dissertation was how susceptibility to two combination products varied among populations and how rapidly resistance and cross resistance developed after one generation of exposure. Surveying the susceptibility of 10 populations of bed bugs to continuous exposures of Temprid SC® uncovered that variation in susceptibility among populations was maintained throughout the duration of exposure. However, continuous exposure to Transport GHP® resulted in 100% mortality of all populations after 14 days (Gordon et al. 2014a). One explanation for the high variation among populations is the extreme bottle neck nearly every population must go through before establishing. The progenitors for most populations are closely realted (e.g.: single mated females; Booth et al. 2012) resulting in extreme founder effects of the subsequent population characterized by limited allelic variation of genes. In addition, other studies have confirmed that the gene flow between populations is also limited (Booth et al. 2012, Saenz et al. 2012, and Fountain et al. 2014). As a result, a PMP can encounter distinct populations that require a unique method to achieve control.

Further exploration into the underlying causes of the resistance and cross

resistance to and among combination products found that the observed decrease in

susceptibility to Temprid SC was mediated by increased resistance to the pyrethroid

component but not the neonicotinoid (Gordon et al. 2014a). This result led to another

research question: can bed bugs develop resistance to the neonicotinoid component in the

combination products? Pyrethroid resistant populations of bed bugs have been well

documented (Romero et al. 2007, Steelman et al. 2008, Yoon et al. 2008, Zhu et al. 2010,

Mamidala et al. 2011), and resistance to the dual action pyrethroid/ neonicotinoid

products has been established in the laboratory (Gordon et al. 2014). My second study

revealed that bed bugs can become resistant to at least one neonicotinoid, imidacloprid,

and that cytochrome P450 mediated detoxification could be one of the underlying causes

of the observed resistance. Given that enzymatic mechanisms of detoxification have

been implicated in cases of cross resistance between insecticidal classes (Devonshire and

Moores 1982, Liu and Yue 2000, Li et al. 2007), cross resistance of the neonicotinoid

selected strain to a pyrethroid was not surprising. Interestingly, cytochrome P450s are

also responsible for activating some proinsecticides (insecticides administered in a non-

active form that require activation endogenously by the insect; Raghavendra et al. 2011),

including the pyrrole contained within Phantom SC®; however, no negative cross

resistance between the neonicotinoid selected strain and Phantom SC was observed here.

This result contrasts with similar experiments that found in *Musca domestica* and

Heliothis virescens increased expression of pyrethroid detoxifying P450s also activated

chlorfenapyr making it more toxic (Pimprale et al. 1997, Sheppard and Joyce 1998).

Results from my study suggest that the specific P450s responsible for insecticide

detoxification in the bed bug are likely not the same ones involved in chlorfenapyr

activation.

To further investigate the role of P450 mediated enhanced metabolism of imidacloprid, gene expression in selected bugs (exposed to neonicotinoids) compared to unselected bugs is being investigated. Messenger RNA has been extracted from groups of bugs from the two treatments (selected and unselected) and complimentary DNA libraries have been constructed and sent to a sequencing lab. The expected genome sequences will then be compared and differences in expression of P450 genes examined. If differences are found in expression, the role of specific P450s will be investigated by using RNA interference (RNAi) to knockdown gene expression in bugs, and then challenging those insects with insecticides. Any observed decrease in resistance upon interfering with expression of a specific P450 would strongly implicate specific enzymes in the observed insecticide resistance.

One characteristic of resistance in a population required for an IRM strategy to work is the existence of fitness costs in resistant individuals relative to susceptible individuals in environments away from the selective agent (Croft 1990). If no costs exist to maintaining the resistance, the frequency of susceptible individuals will not be expected to increase in the absence of the selective pressure. The third study in this dissertation set out to quantify tradeoffs between life history parameters and insecticide resistance. We found that in the absence of Temprid SC, resistant populations were less fecund, had smaller reproductive rates, had a decrease in the proportion of females and had females that senesced sooner relative to susceptible populations with the same origins. The mechanism of the tradeoff could be due to linkage disequilibrium, antagonistic pleiotropy or resource allocation of a finite amount of energy among

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different life history parameters (Stearns 1989, Roff 2002). The next line of research would investigate the hypothesis that increased energy invested in P450 production leads to less resources available to invest into reproduction and ultimately a decrease in fecundity in insecticide resistant populations of *C. lectularius*. Comparing the transcriptome sequences of selected versus unselected bugs that otherwise have the same evolutionary history could identify differential expression of genes involved in enzyme production. RNA interference would then be used to knockdown expression of these genes, then bugs would be challenged with insecticides in an attempt to confirm the role of these enzymes in insecticide resistance. Once the prerequisite experiments identified candidate enzymes involved in insecticide metabolism, F1 offspring of individuals either selected with pyrethroids or unselected will be injected with either control dsRNA (*malE*) or dsRNA coding for genes implicated in insecticide metabolism. Life history tables would then be constructed for three different combinations of treatments: unselected and injected with *malE*, pyrethroid selected and injected with *malE*, and pyrethroid selected and injected with dsRNA coding for detoxifying enzymes. An increase in egg production from the pyrethroid selected strain undergoing RNAi relative to the pyrethroid strain treated with control dsRNA could implicate that an increase in detoxifying enzymes results in a tradeoff between fecundity and insecticide resistance in the bed bug.

In addition to fitness costs, another key factor to an effective IRM strategy is the existence of susceptible alleles within the resistant population. In agricultural systems, migration of susceptible individuals into an area treated with insecticides is a core requirement (Onstad 2008). Fields will often have portions of acreage not treated with insecticides to create refuges that maintain susceptible alleles in close proximity to the

population under selective pressure. Untreated refugia cannot be maintained inside of a residence (the economic threshold for bed bugs being zero), thus migration of new individuals containing susceptible alleles in unlikely (Booth et al. 2012, Saenz et al. 2012, Fountain et al. 2014). In order to manipulate population susceptibility in situations where previous control tactics have failed and selected for resistance, individuals heterozygous for insecticide resistance could be one source of susceptible alleles. If no susceptible alleles remain in the population after treatment, alleviating selection pressure will not result in a reversion toward susceptibility. However, random mating between heterozygous individuals will produce homozygous susceptible individuals, and in the absence of the insecticide, frequency of these individuals should increase and result in a population that once again is susceptible. Thus, understanding the dominance of resistance in these populations would be a critical next step to developing an effective IRM for bed bug control. Traditional IRM relies on using doses of insecticides that would kill rare homozygous resistant individuals and leave no heterozygous individuals (Onstad 2008). However, if resistance is dominant and heterozygous individuals have the same phenotype as homozygous resistant individuals, relieving selection pressure by the same insecticide may allow for a reversion of susceptibility and eventual control.

One final component necessary to develop an effective chemical IRM is the availability of effective products that utilize different modes of action (Onstad 2008). The final research chapter of this dissertation investigated the efficacy of nine commercial products (registered by the Environmental Protection Agency) against six populations of bed bugs with varied evolutionary histories. Results from that study confirmed that populations more recently collected from the field were more resistant

against products in which at least one of the active ingredients was a pyrethroid. However, Phantom SC®, Transport GHP® and CimeXa® were effective at controlling all populations used in the study. This result is promising, because only Transport GHP contains a pyrethroid and neonicotinoid. Phantom SC and CimeXa both contain active ingredients with novel modes of action (pyrrole and desiccant dusts, respectively). Given this information, these three insecticides should be considered first when developing a chemical control plan against an infestation of bed bugs. However, in order to anticipate future control failures, the evolution of resistance to these novel modes of action (electron transport chain disrupter and desiccant dust) needs to be investigated using different strains of bed bugs. Exposing different strains of bugs to residues of an insecticide that are lethal to a high proportion of individuals should increase the frequency of genes conferring resistance. Once a population has reached an established level of resistance, molecular studies can begin elucidating the underlying mechanisms of resistance. Anticipating the rate of evolution and potential molecular mechanisms of any observed resistance will allow for the development of more sophisticated IRM strategies.

Given that a PMP may encounter a population of bed bugs with one of many different resistance profiles, a monitoring kit needs to be created that can establish the efficacy of products before treatments begins. Determining the initial pyrethroid susceptibility of a population allows the PMP to make educated management decisions (Figure 6.1). In cases of pyrethroid susceptibility, a single action pyrethroid or pyrethroid/neonicotinoid product may be effective; however, the use of synergists, products containing novel modes of action or multiple active ingredients stacked in combination with synergists may be necessary to help control populations of bed bugs

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when traditional chemical control methods have previously failed. A test kit would also give the PMPs insight into the changing dynamics of susceptibility in a population and anticipate product failure before it occurs. Being able to prescriptively choose insecticides will not only help control these populations, but it will save pest control companies money by being able to choose the cheapest, effective insecticide and save residents money by requiring fewer treatments to eliminate this pest.

The second step in an effective IRM strategy is the manipulation of resistance in populations of bed bugs by either rotating, stacking insecticidal products, using synergists or any combination of the three (Croft 1990, Bennett 2003, Onstad 2008). When the test kit identifies an issue with resistance, rotation to an alternative mode of action is one option that could relieve selection pressure for insecticide resistance and ultimately control the population. When fitness costs to maintaining resistance exist, alleviating the selection pressure allows a reversion toward susceptibility to occur, assuming susceptible alleles still exist within the population. The reversion means that once resistance to the second mode of action has been selected in an infestation of bed bugs, hopefully, the first mode of action will be effective again. An alternative IRM strategy involves utilizing multiple modes of action at once with the logic that a population can only become resistant to one mode of action at a time. However, rotating between classes of insecticides will only work if the new insecticide does not select for the same mechanism of resistance as the first. Additionally, if resistance to one of the modes of action is already common within the population, that insecticide should be avoided when choosing active ingredients to stack to prevent selecting for populations that can survive exposure to multiple insecticidal classes at one time. A third method of IRM requires at least a

basic knowledge into the molecular mechanism of resistance and utilizes synergists to prolong the efficacy of insecticides once enzyme mediated resistance has been selected. Metabolism can be overcome by the use of chemicals that inhibit enzymes from neutralizing the insecticides before interacting lethally at the target site. Many enzyme inhibitors exist (PBO, DEF, etc.) and are already used in commercial products (e.g: Bedlam® and Drione®), and some lines of research have utilized low doses of ineffective insecticides to synergize the efficacy of other insecticides (Gordon et al. 2012). Understanding the complexities and unique aspects of insecticide resistance as it relates to populations of bed bugs will allow the development and implementation of an IRM strategy targeting populations of bed bugs that will enable the PMP and individuals living with bed bugs to maintain control.

Figure 6.1

Discovery of Bed Bug Infestation — $1200

$500

Evaluate susceptibility/ (mechanism of resistance)

Pyrethroid susceptible

Pyrethroid resistant

Choose a product containing a pyrethroid

Yes

No

Did the pyrethroid control the population?

Is the molecular mechanism of resistance known?

No

Yes

Molecular mechanism of resistance

Metabolic detoxification

Altered target site

Both target site and metabolic mediated

Chemical methods

Use a synergist with a product containing a pyrethroid

Use chlorfenapyr or silica gel

Use chlorfenapyr, synergist, silica gel, and product containing a pyrethroid

Yes

Did chemicals alone control the population?

No

Other methods

Manual removal (steam, encasements, vacuuming)

Fumigate

Heat treatment

Flow chart of a potential bed bug insecticide resistance management (IRM) strategy. Initial treatment chosen based amount of money available for treatment (average price of treatment based on one bedroom apartment; Stedfast and Miller 2014). If the chemical route is chosen, an initial assessment of susceptibility will allow a PMP to choose the most appropriate insecticide. If the initial insecticide fails to control the population, the best IRM strategy should be chosen (considering molecular mechanisms of resistance whenever possible). If all chemical control options fail, use of non-chemical methods (heat, steam, encasements, fumigating, etc.) are recommended.

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cytochrome P450 conferring resistance to pyrethroids in European populations of

pollen beetle, *Meligethes aeneus*. *Ins. Biochem. Molec. Biol.* **45**: 18-29.

VITA

EDUCATION

M.S. Major: Entomology; Concentration: Toxicology. December 2010. Louisiana State University, Baton Rouge, LA. Thesis title: Association of Esterases with Insecticide Resistance in *Culex quinquefasciatus* (Diptera: Culicidae).

B.S. Major: Entomology. May 2008. Purdue University, West Lafayette, Indiana. Capstone project: What Can't a Cricket Do?: A Lesson Plan for Eighth Grade Students.

PROFESSIONAL POSITIONS

Graduate Assistant: August 2008- December 2010. MS student in Entomology, Louisiana State University, Baton Rouge, LA.

Emerald Ash Borer Surveyor: May 2008 – August 2008. Division of Entomology and Plant Pathology, Indiana Department of Natural Resources, Vallonia, Indiana.

G1 Technician: April 2006 – September 2006. USDA-ARS, Purdue University, West Lafayette, Indiana.

SCHOLASTIC AND PROFESSIONAL HONORS

Shripat Kamble Urban Entomology Graduate Student Award for Innovative Research (November 2014). MUVE section award presented at the 62nd Annual Meeting of the Entomological Society of America. Portland, Oregon, $500.

Graduate Student Travel Award (November 2014). University of Kentucky, $400.

First Place Oral Competition (May 2014). Student competition at the National Conference on Urban Entomology. San Antonio, Texas.

National Pest Management Association Intern (October 2013). Chosen to participate in the National Pest Management Association Annual Meeting Pest World 2013. Phoenix, AZ.

President's Prize in Entomology (November 2012). First place in the student competition at the 60th Annual National Meeting of the Entomological Society of America, Knoxville, TN

Monsanto Student Travel Award (November 2012). Money awarded by Monsanto to students and young professionals hosting a symposium at the 60th annual Entomological Association of America annual meeting, $434.

Third Place PhD Oral Paper (October 2012). PhD Student Paper competition at the Ohio Valley Entomological Association annual meeting, Cincinnati, OH, $150.

Honorable Mention (February 2012). American Mosquito Control Association Student Paper competition at the American Mosquito Control Association Annual Meeting, Austin, TX, $500

President's Prize in Entomology (November 2011). First place in the student competition at the 59th Annual National Meeting of the Entomological Society of America, Reno, NV.

Graduate Student Travel Award (November 2011). University of Kentucky, $300.

Clark/Knapp Travel Award (October 2011). University of Kentucky, $150

Urban Entomology Research Fellowship (January 2011). University of Kentucky, $20,000 annually

Louisiana State University Travel Award (December 2010). Louisiana State University Agricultural Center, $280.

Louisiana Mosquito Control Association Research Grant (August 2010). Louisiana Mosquito Control Association, $5,000.

Louisiana State University Travel Award (December 2009). Louisiana State University Agricultural Center, $350.

Lewis T. Graham Graduate Student Research Award (November 2009). Louisiana Mosquito Control Association, $700

George Noffsinger Award (January 2007). POW Pest Management Scholarship, $1,000.

PEER REVIEWED RESEARCH PUBLICATIONS

Gordon, J.R., Goodman, M.H., Potter, M.F. and Haynes, K.F. (2014). Population variation in and selection for insecticide resistance in the bed bug. *Sci Reports.***4**: 3836; DOI: 10.1038/srep03836.

Zhu, F., Gujar, H., **Gordon, J.R.**, Haynes, K.F., Potter, M.F. and Palli, S.R. (2013). Bed bugs evolved unique adaptive strategies to resist insecticides. *Sci Reports.* **3**:1456; DOI:10.1038/srep01456. *Note*: This work gained national media attention with articles in Nature Publishing Group, National Geographic News and UK Ag News.

Gordon, J.R. and Ottea, J.A. (2012). Association of esterases with insecticide resistance in *Culex quinquefasciatus* (Diptera: Culicidae). *J. Econ. Entomol.* **105**: 971-978

PEER REVIEWED OTHER PUBLICATIONS

Gordon, J.R. (2013). Unbiased introduction: The land-grant mission of entomology departments remains economically relevant in the U.S. today. In Abraham, C.M. & Overall, L.M. (Eds.) ESA student debates 2011: Identify, clarify and speak out about the land-grant mission, organic agriculture and host plant resistance programs. *Amer. Entomol.***59**: 217.

Parys, K.A., **Gordon, J.R.**, Brown, S. and Wilson, B. (2013).CON position: Food security for our rapidly growing global human population depends on continued and increased use of insecticides against insect pests of agricultural crops and stored food products. In Burrus, R.G. & Sial, A.A. (eds.) 2009 Student debates: Implications of insect management for human survival. *Amer. Entomol.* **59**:113.

Parys, K.A., **Gordon, J.R.**, Brown, S and Wilson, B. (2012). PRO position: Increasing natural enemy diversity among arthropods is compatible with the goals of biological control and

IPM. In Sail, A.A. and Abraham, C.M. (Eds.) 2010 Student debates: Impact of biological control, transgenic insecticidal crops, and global climate change on arthropod diversity. *Amer. Entomol*. **58**: 96.

TRADE JOURNAL ARTICLES

Potter, M.F., Haynes, K.F., **Gordon, J.R.**, Washburn, L., Washburn, M. and Hardin, T. (2014). Silica gel: A better bed bug desiccant. *Pest Control Technology*. **August 2014**: 76-84.

Gordon, J.R., Goodman, M.H., Haynes, K.F. and Potter, M.F. (2014). Trouble brewing for bed bug insecticides? *Pest Control Technol.* **June 2014**: 72-80.

Potter, M.F., Haynes, K.F., **Gordon, J.R.**, Hardebeck, E. & Arnold, E. (2013). Holy cow… Bat bugs and bird bugs. *Pest Control Technol.* **August 2013**: 72-74, 76, 77.

Potter, M.F., **Gordon, J.R.**, Goodman, M. & Hardin, T. (2013). Mapping bed bug mobility. *Pest Control Technol.* **June 2013**: 72-74, 76, 78, 80

Potter, M.F., Haynes, K.F., **Gordon, J.R.**, Hardebeck, E., and Wickemeyer, W. (2012). Duel-action bed bug killers. *Pest Control Technol.* **March 2012**: 62-76.

INVITED PRESENTATIONS

Dye, K., **Gordon, J.R.**, Crawley, S., Kowles, K., Stamper, C. and Sayeed, A. (2014). From lab to Lexington: Entomology in Action. Poster Presentation. 62nd Annual meeting of the Entomological Association, Portland, OR.

Gordon, J.R. (2012). Molecular mechanisms of insecticide resistance in the bed bug, *Cimex lectularius*. Oral Presentation. Pacific Branch Entomological Society of America Annual Meeting, Portland, OR.

Gordon, J.R., Potter, M.F. and Haynes, K.F. (2011). Insecticide resistance in the bed bug in the laboratory. Poster Presentation. Entomological Society of America Annual Meeting, Reno, NV.

PROFESSIONAL PRESENTATIONS

Gordon, J.R., Potter, M.F. and Haynes, K.F. (2014). Finally some good news: Fitness costs to insecticide resistance in the bed bug. Oral Presentation. 62nd Annual meeting of the Entomological Association, Portland, OR.

Gordon, J.R. (2014). Insecticide resistance in the bed bug. Exit seminar presented to the Department of Entomology at the University of Kentucky, Lexington, KY.

Gordon, J.R., Palli, S.R., Potter, M.F. and Haynes, K.F. (2014). Danger on the horizon: Neonicotinoid resistance in the bed bug. Oral Presentation. National Conference on Urban Entomology, San Antonio, TX.

Gordon, J.R., Potter, M.F. and Haynes, K.F (2013). Potential for the evolution of resistance to neonicotinoids: Oops, they (P450's) did it again. Oral Presentation.

Entomological Association of America Annual Meeting, Austin, TX.

Gordon, J.R. (2013). Bed bug update. Oral Presentation. University of Kentucky Pest Control Short Course, Lexington, KY.

Gordon, J.R. (2012). Evolution of resistance to combination insecticide products in the bed bug. Oral Presentation. Entomological Association of America Annual Meeting, Knoxville, TN.

Gordon, J.R. (2012). Evolution of insecticide resistance in the bed bug. Oral Presentation. Ohio Valley Entomological Association Annual Meeting, Cincinnati, OH.

Gordon, J.R. and Ottea, J.A. (2012). Association of esterases in resistance to adulticides in field-collections of the Southern house mosquito. Oral Presentation. American Mosquito Control Association Annual Meeting, Austin, TX.

Gordon, J.R. Potter, M.F. and Haynes, K.F. (2012). Insecticide resistance in *Cimex lectularius* in the laboratory. Poster Presentation. American Mosquito Control Association Annual Meeting, Austin, TX.

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Gordon, J.R., Potter, M.F. and Haynes, K.F. (2011). Insecticide resistance in the bed bug: An evolving story. Oral Presentation. Entomological Society of America Annual Meeting, Reno, NV.

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