To my loving and supportive family
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Residual insecticides have been extremely important in reducing pest pressures and disease transmission from insect pests, and are largely effective due to long-lasting active properties. Currently, polymers are used in residual insecticides to maintain activity and reduce break down of the application. However, little is known about how well a formulation with a slow-release polymer will function when a sorptive dust is added into the solution. Amorphous silica is a type of sportive dust, and is presently used as such in dust formulation insecticide products. The incorporation of different concentrations of silica and polymer into a residual spray formulation was evaluated to determine potential benefits associated with the use of these materials against various insect pest species. Increasing the concentration of silica in formulation resulted in faster times to knockdown, whereas increasing concentrations of polymer reduced time to knockdown. Aging of these formulations revealed that increasing concentrations of polymer did not help to maintain the residual activity of the application. Similarly, with increasing concentrations of polymer, the less effective the treatment was after weathering in an artificial rain event. The greater the concentration of silica, however, the more effective the formulation was both before and after the rain event. Using silica
and polymer in formulation reduced the time to knockdown when combined with indoxacarb, chlorantraniliprole and chlorfenapyr. Additionally, the use of silica and polymer in formulation did not affect efficacy of a trap used to control fruit flies, but the use of a bait in conjunction with the treated trap significantly improved trap efficacy.
The use of residual insecticides has been important and necessary for the control of many pest insects, particularly ones that transmit disease-causing organisms (Najera and Zaim 2001). For instance, global malaria control is dependent on residual spraying to reduce cases of transmission (Pluess et al. 2010). In 2015, approximately 212,000,000 cases and 429,000 deaths from malaria were reported (WHO 2016); according to the World Health Organization (WHO), this a 29% reduction since 2010. The decrease in cases and deaths is attributed to prevention and control measures, which is largely comprised of residual insecticide spraying (Najera and Zaim 2001, WHO 2016). The primary advantages of residual insecticide formulations are their longstanding activity and stabilization of active components when under conditions of environmental degradation (Tsuji 2000). In addition to maintaining the activity of insecticide applications, the use of residual formulations lessens the migration of biologically active constituents from the area of application and reduces both toxicity and objectionable non-target impacts (Bahadir and Pfister 1990, Wilkins 2003).

Residual insecticides that include a polymer are classified as controlled-, or slow-release insecticides (Bahadir and Pfister 1990). In these formulations, the active ingredients (AIs) are chemically or physically bound in a polymeric matrix. Incorporation of AI and polymer is achieved through different techniques, but ultimately results in a particle or film formulation from which the active component is released (Scher 1999). Coatings and carriers that are made from biodegradable materials that retain the aforementioned properties are the focus of a growing body of research. In recent years, a variety of polymeric materials have been investigated for their attributes and
characteristics that influence delivery and release rate of chemicals (Voinova 2008). Examples of these types of materials that have been studied for their efficacy as pesticide carriers include: 1) polyurethane, 2) ethyl cellulose, 3) methacrylate, and 4) sodium alginate (Kulkarni et al. 2000, Perez-Martinez et al. 2001, Shukla et al. 2002, Piletska et al. 2005).

Silica gel is another material that has been largely explored in pesticide research and development for its beneficial properties. Often used in the pest management industry as a dust formulation, products containing silica gel have been growing in popularity. Having superior lipid-absorbing qualities, this compound works by removing the protective waxy layer on the outside of an insect’s cuticle (Potter 2014). Without their lipid layer, insects lose the ability to retain moisture, ultimately resulting in death by desiccation (Ebeling 1961).

With a considerable increase in cases of pesticide resistance occurring all over the world, formulations that operate as a desiccant could be favorable. This predilection is suggested because it is unlikely for insects to evolve resistance to desiccant insecticides (Potter 2014). Mutation-selection and changes in gene frequencies provide the platform for the modern theory of evolution, and are the processes that give rise to insecticide resistance (Tuskamoto 1969). Though a variety of suggested mechanisms exist, a widely accepted and documented explanation for insecticide resistance is the upregulation and metabolism of toxic compounds (Oppenooirth 1965, Tuskamoto 1969, Plapp 1976). According to the WHO, resistance to the top four insecticide chemical classes has been recorded in the analysis of every malaria vector (WHO 2015). Extensive literature is available on insecticide resistance occurring in a wide-range of
medically and economically important pest species, including, but not limited to: 1) German cockroaches, 2) house flies, 3) fruit flies, 4) bed bugs, and 5) mosquitoes (Scott et al. 1990, Morton 1993, Hemingway 2000, Liu and Yue 2000, Zhu et al. 2010, Vontas et al. 2012).

The negative impacts from insecticide resistance on human health and the environment are vast. More pesticide is often required to achieve control of resistant populations (Morton 1993). Coupling an active ingredient with a desiccant and polymer could provide a more effective residual insecticide; the investigation of this idea is the primary goal of this research. By combining these ingredients, an insect is targeted with two distinctive modes of action. In addition to this, a residual polymeric formulation containing silica would increase the surface area of an application. Understanding the potential synergistic effects of these materials is critical for designing more effective control tools. The objectives of this research were to: 1) evaluate different concentrations of silica and polymer within a residual insecticide formulation against major insect pest species, 2) understand the effects of aging and weathering on these formulations, 3) evaluate the incorporation of silica and polymer into formulations containing various active ingredients, and 4) evaluate the use of formulations containing silica and polymer when used in combination with an attractive bait. Due to the diversity in biology, behavior and morphology of top destructive and nuisance insect pests, a total of 8 species were used in evaluating formulations: 1) *Blattella germanica* L., 2) *Cimex lectularius* L., 3) *Drosophila repleta*, 4) *Drosophila melanogaster*, 5) *Camponotus floridanus*, 6) *Aedes aegypti*, 7) *Musca domestica*, and 8) *Solenopsis invicta*. The hypotheses to be tested were: 1) the use of silica and polymer would improve the
efficacy of the residual insecticide formulations, 2) formulations containing silica and polymer would remain effective under aging and weathering conditions, 3) silica and polymer would improve the efficacy when used with a variety of active ingredients, and 4) formulations containing silica and polymer would be effective when used in combination with a bait.

Testing the formulations containing the different concentrations of silica and polymer, the aging and weathering tests, and the tests done to evaluate the ingredients when used with different active ingredients were all conducted in laboratory forced exposure assays. The use of these materials in combination with a bait was evaluated in an open arena assay.
CHAPTER 2
LITERATURE REVIEW

German Cockroaches

Biology and Importance

The German cockroach (*Blattella germanica*) is a medically and economically important pest species that has strongly adapted to living in close association with humans (Gondhalekar 2011). This synanthropic pest is the most abundant of all cockroach species and has a cosmopolitan distribution (Schal and Hamilton 1990). Its generalistic and opportunistic feeding behaviors contribute to their survivability and overall success as a major household pest (Jones and Raubenheimer 2000). Pervasive surveying of home owners and pest control operators has affirmed that cockroaches are the primary pest of concern, with particular emphasis on the German cockroach (Schal and Hamilton 1990, Robinson and Zungoli 1985).

The great proclivity for *B. germanica* infestations amplifies threat to homeowners, commericials kitchens, restaurants, hotels, food production facilities, and grocers (Rust et al. 1995). *B. germanica* is the most common cockroach species collected from restaurants, comprising 96% of the cockroach samples (Zhai and Robinson 1991). Their ability to mechanically transmit disease-causing microorganisms makes them more than just a nuisance pest, but also a public health concern (Holakuei et al. 2004). A myriad of parasites, fungi, bacteria and viruses have been isolated from field-collected German cockroaches (Hamu et al. 2014). In addition to the risks associated with disease transmission, German cockroaches are also known to produce allergens that can lead to asthma attacks, hypersensitivity reactions, and perennial rhinitis (JiingGuang et al. 2010).
Control Methods

High reproductive output, rapid population growth, harborage-seeking behaviors, lack of natural domestic predators, and preferred habitats, which are often around food-handling areas, are a few reasons why accomplishing German cockroach control can be difficult. Conventional active control methods include toxic bait stations, toxic gel baits, crack-and-crevasse treatments, aerosol sprays, dusting, and residual pesticide application sprays onto areas likely to be traveled over by German cockroaches (Koehler et al. 1996, Ebeling 1975).

A variety of active ingredients have been used in bait formulations to manage German cockroach infestations. Cockroach baits have been formulated with active ingredients from the carbamate, oxadiazine, organophosphate, phenylpyrazole, neonicotinoid and chlorinated hydrocarbon chemical classes (Appel 1990, Rust et al. 1995). The major drawbacks associated with the use of toxic baits are bait aversion and repellency (Appel 1990). Allethrin, permethrin, cyfluthrin, propoxur, malathion, chlorpyrifos, lindane, DDT, and chlordane are some active ingredients used in other products and formulations that target B. germanica. However, some of these chemicals are no longer permitted for use in the United States. Indoxacarb, fipronil and dinotefuran have been popular in recent years for German cockroach control. Resistance has been well documented for occurring within B. germanica populations globally, further exacerbating the toil for control (Cochran 1991, Metcalf 1980, Rust and Reiersen 1978, Scott et al. 1990).
Carpenter Ants

Biology and Importance

Carpenter ants (*Camponotus* spp.) are considered an economically important pest throughout the United States because of the structural damage they cause (Gibson and Scott 1989, Mankowski and Morrell 2011, Wood 1989). These ants do not feed on wood, but will excavate galleries in wooden structures to colonize. Though carpenter ants commonly infest marred wood, the creation of galleries can further compromise the overall structural integrity of the infested woodwork. As the colony grows, satellite colonies will form and extend into other areas of the structure. Nests can contain upwards of 6,000 workers and can have as many as five mating swarms over the course of 3-5 months (Fowler 1986). Infestations and damage have been observed in heartwood, decaying and seasoned wood, wall voids, and insulation (Fowler 1986). Nests have also been located in garage castings, support beams, roofs, window frames, studs, siding, and porch pillars of homes (Hansen and Klotz 2005).

Control and Importance

Evidence of ants, alates, wood dust, or noise from ant activity in the walls are indicators of carpenter ant presence in a home. If ant activity, presence and damage are evident, homeowners can request either a partial (spot) treatment, or a targeted complete treatment. A complete treatment would involve source identification, residual spraying, and baiting. Locating the nest allows for treatment at the source, ultimately yielding full control of the pest. Spot treatments would be more localized residual sprays that target foraging workers; this method would not have a great impact on a large, established colony, thus resulting in little to no control (Fowler 1986). However, spot treatments may be effective against localized or new carpenter ant infestations (Hansen
and Klotz 2005). Dusting to the wall voids, studs and sheet insulation during the construction phase will help prevent carpenter ant infestations and provide years of residual activity and protection (Hansen and Klotz 2005). Perimeter sprays applied at the foundation around the home can serve as a protective barrier against carpenter ants.

Attractive toxic baits can be a suitable alternative to pesticide applications, but do have limitations with foraging seasons. This method usually takes time to control the ants, as the toxicant must disseminate throughout the entire colony (Tripp et al. 2000). Active ingredients from several chemicals classes have been used in formulations against carpenter ants: organophosphates (diazinon, chlorpyrifos, dimethoate and malathion), carbamates (bendiocarb, carbofuran, and propoxur), phenylpyrazoles (fipronil), and pyrethroids (deltamethrin, permethrin, pyrenone, and fenvalerate). Deltamethrin and diazinon have been shown to have the greatest toxicity to carpenter ants in a study that evaluated 14 active ingredients (Gibson and Scott 1989). Presently, fipronil, abamectin and deltamethrin are popular active ingredients used to control carpenter ants.

**Fire Ants**

**Biology and Importance**

The red imported fire ant (*Solenopsis invicta*), or RIFA, is considered to be the most economically and ecologically destructive ant species in the United States (Allen et al. 2004). The RIFA is an invasive ant pest that has profoundly and rapidly expanded throughout the southeastern United States since its introduction in the 1930’s (Lofgren et al. 1975). High reproductive capacity and lack of natural predators are characteristics that have allowed the red imported fire ant to flourish. One year after colony
establishment, a nest can have approximately 11,000 workers. Two to three years after establishment, worker count can increase to over 230,000 (Vinson and Greenberg 1986). With an estimated spread rate of $1.47 \times 10^5$ ha per year, this species has successfully established colonies in well over 100 billion hectares in the United States (Callcott and Collins 1996). The RIFA commonly occurs in disturbed habitats, such as pastures, yards, groves, golf courses, agricultural plots, and roadsides. The mounds they construct, their aggressive behavior to defend nests, and tendency to inflict painful, sometimes life-threatening, stings has made the red imported fire ant an urban, recreational and agricultural pest (Lofgren et al. 1975). A national annual economic impact value of more than $6.5 billion has been estimated in the US for damages and losses related to *S. invicta* (Pereira et al. 2002).

Ecological consequences have also arisen as a result of RIFA’s aggressive nature. The dominance of this ant has dramatically altered densities of native ants in the southern states, ultimately outcompeting and displacing them (Gotelli and Arnett 2000). In addition to the impact *Solenopsis invicta* has on arthropod communities, native vertebrates are also highly vulnerable to RIFA invasion and inundation (Allen et al. 2004). It is documented that vertebrate populations, such as rabbit, squirrel, snake, wood duck, cliff swallow and quail, have decreased in areas where RIFA remain uncontrolled (Allen et al. 1994, 1995, Tschinkel 2006). The negative impacts on the biodiversity of animal communities where these ants thrive, in conjunction with the nuisance of ant mounds and medical importance associated with stings, demonstrates the need for *S. invicta* control.
Control Methods

By the 1960’s, the United States called for federal and state eradication programs in attempts to eliminate *S. invicta* populations and cease spread (Lofgren et al. 1975, Kaiser 1978). During this same period, toxic baits for fire ants were developed and provided good RIFA control. The active ingredients used in these early baits against *S. invicta*, such as Allied Chemical GC-1 189 (dodecachlorooctahydro-1,3,4-metheno-2H-cycJobuta[cd]pentalene-2-one) (Kepone®) and mirex, were found to have dangerous attributes like high mammalian toxicity, bio-accumulative properties, and negative non-target species impacts (Lofgren et al. 1975, Kaiser 1978, Alley 1973). Use of these products has been prohibited and newer baits have since been developed. Active ingredients such as indoxacarb, spinosad, hydramethylnon, fipronil, methoprene, fenoxycarb, and abamectin have been used in various bait formulations (Oi and Oi 2006, Barr et al. 2005). Baits take time to disseminate throughout the colony, but are highly effective in gaining overall control of nests. An important attribute of active ingredients used in baits is to be slow acting so it has time to be passed along to all the members via trophallactic feeding behaviors before eliciting toxic effects. Using contact insecticides may provide more immediate control of RIFA nests, but is not as effective in gaining complete populational control.

Bed Bugs

Biology and Importance

Bed bugs (*Cimex lectularius*) are classified as a significant pest of public health importance in the Pesticide Registration Notice 2002-1 – an article produced by the collaborative efforts of the Environmental Protection Agency (EPA), the United States Department of Health and Human Services (HHS) and the United States Department of
Agriculture (USDA). Recent resurgence of \textit{C. lectularius} has been experienced on a global scale (Potter 2011, Doggett et al. 2004, Boase 2001). The status of this comeback in the United States can be seen in the data collected by the NYC Department of Housing Preservation and Development, which showed that bed bug complaints increased 20-fold over the course of a 5-year study – a 10,448 annual complaint increase from 2004 to 2009 (Bloom et al. 2010). In Australia, a 4,500% increase in cases of infestation from 2000 to 2006 was documented (Doggett et al. 2011).

These ectoparasites have been a nuisance to, and evolved closely with, humans since the Paleolithic Age (Potter 2011, Usinger 1966). As a result of their small size, nocturnal feeding behavior, and cryptic nature, detection is often difficult. Control is not usually sought until after a population has already been well established. A single female bed bug can produce upwards of 500 eggs in her lifetime, laying ~5 eggs per day (Delaunay et al. 2011, Pereira et al. 2012). The above behavioral and biological characteristics, in conjunction with frequently delayed control, raises the infestation potential. Prone to clinging onto clothes, furniture and luggage, bed bug dissemination through increased travel has further confounded the problems associated with achieving control.

Though bed bugs are hematophagous and will readily feed upon humans, they are not known to transmit any disease-causing organisms (Boase 2001, Delaunay et al. 2011). Albeit disease transmission is not presently a concern associated with bed bugs, the dermatological and psychological damages that ensue due to feeding are enough to place \textit{C. lectularius} as a serious urban pest. Allergic reactions, irritation, cutaneous
inflammation, lesions, and secondary infections from bacteria entering into broken skin caused by incessant itching are not uncommon manifestations of bed bug bites (Usinger 1966, Leverkus et al. 2006, Goddard et al. 2011). Extreme cases of bed bug infestations have even resulted in anemia (Pritchard and Hwang 2009). The great discomfort and anxiety generated by bed bug bites is a major concern for hotels, nursing homes, university dormitories, hospitals, apartment complexes, and military camps, housing and warships. These types of facilities will usually have strict bed bug monitoring and extermination policies delineated.

**Control Methods**

Some early bed bug management measures are presently considered nonsensical or dangerous: hanging the feet of dead animals on bed posts, fumigating with cyanide or burning sulfur, and using substances like gasoline or mercury chloride (Usinger 1966, Potter 2011). It was not until the early 1940’s that the United States began to adequately control bed bugs through the use of dichloro-diphenyl trichloroethane (DDT) (Usinger 1966, Potter 2011). The effectiveness of DDT lead many to believe that this would be the solution to completely eliminate bed bugs. But with DDT resistance documented by 1947, the success was ephemeral (Usinger 1966, Potter 2011). Recommendations by the National Pest Control Association at that time were to use alternative chemicals. Popular insecticides used throughout the 1950’s-1970’s included products that contained malathion, lindane, diazinon, or chlordane (Potter 2011). Dust and spray applications with these products comprised of treating mattresses, bedframes, baseboards and in the cracks or crevasses around sleeping areas. Though many of these chemicals belong to classes that are no longer permitted for use in the United States, these same application techniques are still employed with
current registered products. Presently, pyrethroids, such as deltamethrin, lambda-cyhalothrin, and permethrin, are the preferred active ingredients used by pest control operators for bed bug treatments (Gangloff-Kaufman et al. 2006). As a result of this overwhelming use, however, pyrethroid-resistant populations of bed bugs have become prevalent globally (Boase et al. 2006, Romero et al. 2007, Yoon et al. 2008, and Lilly et al. 2009). In attempts to address resistance issues, combination products containing two active ingredients have been popular in recent years. Insecticides containing imidacloprid and cyclopropanecarboxylate, or acetamiprid and bifenthrin, are examples of combination products currently in use.

In addition to the traditional approaches for controlling bed bugs, some pest management companies offer heat treatments and fumigation to combat infestations. These techniques effectively kill all life stages. Furthermore, consumers have access to a wide range of products marketed to protect consumers from, monitor for, and reduce the likelihood of bed bug infestations. Readily-available products include mattress encasements, treated mattress liners, lure traps, active and passive interceptor traps, foggers, dusts and soap solutions. The public’s negative perception of bed bugs, the rapid development of pesticide resistance, and the global increase in incidences all collaboratively demonstrate the pressing need for rigid control methods (Pottery 2011).

**House Flies**

**Biology and Importance**

House flies (*Musca domestica*) are a major pest to both humans and animals, particularly livestock. Suitable breeding and oviposition substrates include horse, chicken, dog, goat and cow manure, decaying vegetable matter, kitchen waste, grass clippings, decomposing animal matter, and moist areas around hay, grains or troughs.
(Bishopp et al. 1915). Such variety of sufficient egg-laying habitats leads to pest pressure in both rural and urban landscapes.

The severe stress and annoyance experienced by livestock and workers from house flies have grievous economic implications. Stress and irritation from arthropod presence compromises health by reducing egg or milk production in livestock (Steelman 1976). House flies are the most important pest species to control in poultry facilities (Chapman et al. 1998). The poultry industry spends an estimated $20 million on insecticide applications alone to suppress fly nuisance annually (Geden and Hogsette 1994).

More than a nuisance pest, house flies are a substantial public health threat due to their ability to mechanically transmit over 100 human and animal disease-causing organisms. These pathogens include viruses, rickettsia, bacteria that cause salmonella, typhoid fever, cholera, and tuberculosis, protozoans that cause dysentery, and an array of helminths such as pinworms, roundworms and tape worms (Scott et al. 2009). House flies are also effective disseminators of virulent antibiotic-resistant bacteria (Macovei and Zurek 2006). Many attributes of this organism contribute to its strong ability to transmit the extensive list of pathogens: ability to travel long distances, rapid population development, the attraction to decomposing organic matter, including feces and carcasses, and their affinity to humans and their food.

Control Methods

House flies are controlled through a variety of both active and passive measures. Active measures include contact and residual pesticide applications, insecticide-laced bait mixtures, treated bands or chords, and larviciding manure (Axtell 1970). Passive, or physical, control measures include sticky tapes, light traps and electrocutors. In many
cases, control is accomplished through the integration of multiple approaches known as Integrated Pest Management (IPM). Often times, source-reduction and environmental sanitation practices will greatly reduce the density and intensity of house fly populations in problem areas (WHO 1986). Clean farms will often be bypassed by flies in order to access locations of greater filth (Pickens et al. 1967).

Similar to most pest insects during the 1940’s - 1960’s, *Musca domestica* control was well-achieved with the use of DDT. With the successful control provided by this chemical, headlines such as “No Flies in Iowa” (*Time Magazine*, 1947) and “Entire Towns Abolish Flies” (*Reader’s Digest*, 1948) were common (Metcalf 1980). Soon after major environmental concerns arose from the wide-spread use of DDT, pressure for the development of alternative chemicals intensified. Active ingredients within other insecticide chemical classes have since been produced and used against this pest, such as organophosphates (diazinon, rotenone, bromophos, coumaphos, fenthion, fenchlorphos, iodofenphos, dimethoate, malathion, trichlorfon, dichlorvos, and tetrachlorvinphos), organochlorines (lindane, dieldrin, and chlordane), carbamates (dioxacarb, bendiocarb, methomyl, propoxur, and dimetilan), pyrethroids (pyrethrins, tetramethrin, and decamethrin), and juvenile hormone analogues (methoprene) (WHO 1986, Metcalf 1980). Currently, cyantraniliprole, methomyl, dinotefuran, and imidicloprid are popular active ingredients used to control house flies.

**Fruit Flies**

**Biology and Importance**

Members of the *Drosophila* fruit fly genus receive more attention in the scientific realm for their significant role in genomic studies, but this group of flies are pests nonetheless. Since these fruit flies are not a considerable threat to humans or crops,
*Drosophila melanogaster* and *Drosophila repleta* are not classified as pests of significant public health or agricultural importance according to the Pesticide Registration Notice 2002-1 and the Regulated Plant Pest List produced by the USDA. Frequently developing in moist areas around kitchens, *D. melanogaster* and *D. repleta* are predominantly considered nuisance pests to homeowners. These domestic flies are highly attracted to over-ripe fruits and decaying organic matter, and are often introduced into homes on these grocery goods (Hill 1997). Fly management concerns do exist in areas where produce is handled, shipped, and sold. Since these flies are not a damaging threat to marketable fruit, the concern for management is centered around the annoyance associated with fly presence, societal perception of uncleanliness, and disease transmission. Mechanical transmission of pathogens was identified in a study that collected *Drosophila* flies from a market in New Orleans. *Salmonella typhi* was isolated from the *Drosophila sp.*, demonstrating their potential to spread diseases (Beyer 1925, Greenberg 1973).

Fruit flies have nearly global distribution, persisting on all continents except Antarctica. Distribution patterns of *Drosophila* expands through a wide range of climactic zones, demonstrating their extensive adaptive capacity (Hangartner et al. 2015). Warmer temperatures and higher moisture levels are suitable for offspring to develop and thrive. Areas with these qualities, such as drains or catch pans under refrigerators, are oviposition sites in homes. A female fruit fly can lay 50-70 eggs per day. With a rapid development time of 8 to 10 days to fully mature, populations can become dense if uncontrolled. Upon initial inspection, *D. melanogaster* and *D. repleta* adults look very similar. The two species only vary slightly morphologically: *D.*
*melanogaster* is slightly smaller, weighing 3 mg as an adult, and will often have distinct red eyes, whereas *D. repleta* weighs approximately 3.5 mg and will typically have a darker eye color (Greenberg 1973).

**Control Methods**

Good sanitation practices and screening measures can help to protect food from fruit fly contamination and development. Management techniques implemented by both grower and processor have shown to reduce the likelihood of fruit fly infestations occurring in facilities, markets or consumer homes. Insecticide treatments have been used on crops in the field and on crates of harvested produce to reduce *Drosophila* presence (Pepper et al. 1953). Aerial applications to fields for fruit fly control has also been employed.

Active ingredients such as DDT, permethrin, malathion, diazinon, methoprene, dichlorvos and naled have been used in formulations like bait, emulsifiable concentrates, fly strips, wettable powders, aerosols and granular products targeting *Drosophila* flies (Davis 1960, Yerington 1967). Presently, pest control companies express the difficulty to gain control over fruit fly populations and emphasize the importance of source identification and elimination to achieve management. Recommendations like bacterial digesters to clean drains, or attractive fruit fly traps that lure in and capture flies, are often suggested to homeowners seeking relief from *Drosophila* nuisance.

**Aedes Mosquitoes**

**Biology and Importance**

Due to their classification as a significant threat to public health, millions of dollars are spent annually to control mosquitoes in Florida. Lee County mosquito control
district alone has a $16 million annual operational budget (Dennis and Sun 2016). Mosquito control is largely addressed at federal and state levels, especially during disease outbreaks. Stringent population abatement plans are created and implemented through the cooperative efforts of major federal agencies, including the Environmental Protection Agency, United States Department of Health & Human Services, and the United States Department of Agriculture (EPA Notice 2002-1). Species within the Aedes genus have made headlines in recent years for their role in transmitting arboviruses such as Chikungunya, Dengue, and Zika (Sathantriphop et al. 2015, Nuckols et al. 2015, Ponlawat and Harrington 2005). These mosquitoes are also competent vectors of several encephalitic viruses and filarial parasites, such as Dirofilaria immitis (dog heartworm) (Cancrini et al. 2003, Paupy et al. 2009, Moore and Mitchell 1997). In 2014, American philanthropist Bill Gates, in attempts to increase public awareness, illuminated the magnitude of danger ascribed to mosquitoes by dubbing the insect “The Deadliest Animal in the World” (Gates 2014).

In Florida, Aedes albopictus and Aedes aegypti are the two main species of concern. Found on all continents except Antarctica, Ae. aegypti thrive in the climactic zone between 45˚N and 35˚S latitudes (Simard et al. 2005). The Asian tiger mosquito (Ae. albopictus), is an invasive pest of Asian origin that has successfully established throughout the Americas, Europe, Africa, and Australia (Kobayashi et al. 2002, Foley et al. 1997, Ogata and Samayoa 1996, Savage et al. 1992). In North America specifically, this mosquito is strongly concentrated in the southeastern continental United States (Moore and Mitchell 1997).
Though *Ae. albopictus* and *Ae. aegypti* are considered container breeding mosquitoes, habitation and oviposition preferences do vary slightly between the two species. Suburban to rural habitats have shown to provide more favorable development sites for *Ae. albopictus* larvae, but adults can be found in both forested and urban landscapes (Deing et al. 2001, Braks et al. 2003). These mosquitoes will deposit eggs into organically rich tree holes and other natural areas that allow for water collection (Deing et al. 2001). Egg survivability and viability remains strong for months after oviposition (Christophers 1960). This desiccation-resistant trait in *Aedes* eggs contributes to their resiliency through dry seasons (Russell et al. 2001). *Aedes aegypti* populations exist predominantly in more urbanized areas and will commonly utilize containers, such as jugs, tires or other artificial receptacles, to deposit eggs (Christophers 1960). These containers are often most abundant around homes, thus contributing to the greater densities of *Ae. aegypti* around humans (Vontas et al. 2012). In areas where populations overlap, both larval species can be found co-occurring in the same habitat (Braks et al. 2003). Humans exacerbate *Aedes* prevalence through the provision of conducive artificial egg-laying habitats and promote the ever increasing global distribution patterns through travel. These occurrences, in conjunction with acute medical importance, emphasizes the need for control of these two major arbovirus vectors.

**Control Methods**

Mosquito control is broadly achieved through targeting both the larval and adult life stages in an integrated approach. Since mosquitoes pose such great threat to human and economic health, state and local agencies are responsible for the creation and implementation of mosquito control programs. In Florida specifically, there are a
total of 61 mosquito control districts. Each district aims to safeguard the public in their areas through strict surveillance, inspections, insecticide applications and pathogen screening techniques to identify outbreaks. As reported by the Florida Department of Agriculture and Consumer Services (FDACS), aerial insecticide applications occur across the state, but ultra-low volume (ULV) sprays are more widely executed through the use of ground equipment. Ground applications are typically accomplished with truck-mounted sprayers. ULV sprays disperse as micro-droplets that target mosquitoes in flight. According to the United States Environmental Protection Agency, common active ingredients used in ULV applications are malathion and naled in the organophosphate chemical class, and permethrin, sumithrin, and resmethrin in the pyrethroid chemical class.

Insecticide resistance in mosquito populations has generated substantial challenges in efforts to gain control. *Aedes* mosquitoes have developed resistance to major chemical classes including organophosphates, pyrethroids, and carbamates (Rodriguez et al. 2001, Vontas 2012, Harris et al. 2010). This can largely be attributed to the extensive and wide-spread use of popular active ingredients. Some organophosphates, such as malathion for example, have been registered with the EPA since the 1950’s and are still currently employed for use against mosquitoes (EPA 2010).

In addition to resistant populations, mosquitoes have displayed avoidance behaviors to residual surface treatments, which is an alternative adulticidal application to ULV sprays (Kongmee et al. 2004, Polsomboom et al. 2008). Resistance and avoidance behaviors exhibited by these mosquitoes intensifies the need for alternative
management strategies. Since vector control is the primary method to reduce disease transmission, proactive population monitoring is necessary to ensure that control tactics are effective. Attractive, lethal and gravid traps, space repellents and insecticide-treated materials (ITM) are available for public use and are good for increasing personal protection. Large-scale mosquito management success can be accomplished through source elimination as homeowners become more aware of the importance of removing larval habitats (Morrison et al. 2008).

**Polymers and Silica in Insecticides**

**Polymers**

The use of polymers, such as waxes, resins, varnishes and urea-formaldehyde polymers, began to be investigated in the 1960’s for their advantageous properties when incorporated into insecticides (Price 1960, Medley and Drummond 1962, Lloyd and Maithysse 1966). A polymer-insecticide combination remained effective against insect pests for years after application, with some resin-type films maintaining high activity after 6 years (Price 1960). Active ingredients integrated into plasticized polymeric matrices are referred to as controlled-release insecticides (Cardarelli and Cardarelli 1982). Beneficial properties related to the incorporation of an insecticide into polymeric nanoparticles, beads and films for increased active longevity have also been claimed in United States Patents (Seiner 1972, Wysong 1984, Farquharson et al. 1989, Liebert et al. 2000).

Variations in polymer nanoparticle size, type, method of delivery, and flexibility affect the rate of insecticidal elution (Lloyd and Maithysse 1966). Depending on the controlled-release mechanism, active agents can discharge through several methods. Examples of mechanisms used include: a) nanocapsule/micro-encapsulation where the
active component is bound within a polymeric envelope, b) pendent substitution where active ingredient is cleaved to a polymer backbone, and c) nonporous, monolithic polymeric film that allows for insecticidal migration through an applied layer (Rao and Geckeler 2011, Cardarelli and Cardarelli 1982, Von Kohorn et al. 1987). Coupling chemical agents to a polymeric structure not only increases dispensation control, but has also shown to inhibit volatilization and reduce degradation while preventing loss from leeching (Takei et al. 2008).

**Silica**

The use and incorporation of amorphous silica into dust formulations to control pests has been studied since the 1960’s (Shawir et al. 1988, Kane 1967, Le Patourel and Singh 1984, Ebeling 1971, Schultz et al. 2014). When used as a powder formulation, synthetic silica gel acts as a desiccant by absorbing lipids from the cuticular layer, causing insects to dry out from moisture loss (Ebeling 1971, Barik et al. 2008). However, very little information is available on the effects of silica in a residual spray formulation. Potter (2014) compared the efficacy of an amorphous silica dust application to an application of amorphous silica in an aqueous solution. The empirical data produced from that study showed that the solution spray application was significantly less effective than the dust application in reducing the number of bed bugs in an infested apartment. However, the amount of silica gel used in solution was far less than what the label rate for liquid application called for.

A more recent study on termites found that silica type and characteristics, such as surface area and mesoporous structure, influenced insecticidal release and adsorption capacity (Popat et al. 2012). Insecticidal effectiveness and improvement with the use of silica both alone and in solution is claimed in patents (Boase et al. 1986, Vrba
1992), but scientific literature examining this is insufficient. An explanation of potentially enhanced toxicity of a dust insecticide formulated with silica was proposed by Le Patourel and Singh in 1984. These researchers suggested that when insects have lost some of the protective lipid layer of the cuticle, they could become more vulnerable to the effects of an insecticidal chemical.

Current products on the United States insecticide market containing silica include Tri-Die dust, CimeXa insecticide dust, and Drione desiccant dust. CimeXa dust contains 100% amorphous silica, while the other 2 products are formulated using pyrethroid active ingredients in conjunction with silica gel. Unlike naturally occurring crystalline silica, synthetic, non-crystalline silica gel is relatively non-toxic to mammals (Potter 2014). With a similar LD50 to that of table salt, there are no inhalation hazards associated with non-crystallized silica gels.
CHAPTER 3
MATERIALS AND METHODS

Insect Rearing and Handling

All insects used in this study were obtained from laboratory colonies. *B. germanica* L., *C. lectularius* L., *D. repleta*, *D. melanogaster*, *C. floridanus*, *Ae. aegypti*, and *S. invicta* colonies are maintained in the Urban Entomology Laboratory located at the University of Florida in Gainesville, Florida. *M. domestica* pupae were provided by the United States Department of Agriculture, Agriculture Research Service (USDA-ARS) in Gainesville, Florida and adults were maintained in the Urban Entomology Laboratory.

German Cockroaches

Orlando German cockroaches (*B. germanica*) were a nonresistant “normal” strain that was been kept in colony at the University of Florida since the 1950’s. These cockroaches were originally provided by the USDA and were maintained according to the rearing procedures described in Koehler and Patterson (1986). Laboratory colonies remained in the rearing room under the following controlled conditions: approximately 26°C, 55% RH and 12:12 h (L:D) photoperiod. German cockroach colonies were kept in glass containers (25 cm height by 22.5 cm diameter) covered with a cloth lid, which was secured by a rubber band. The roaches could seek refugium within cardboard harborages, were provided water and fed with dry dog food (Pedigree® Puppy, Mars Inc., McLean, VA). Cockroaches used in the study were picked out of the colony containers and put directly into the laboratory bioassays without sedation.

Ants

The carpenter ant workers used in these bioassays were from a colony that has been maintained at the University of Florida since 2011. *Solenopsis invicta* workers
used in these bioassays are from a colony established with a queen that was collected in 2015 in Gainesville, FL. Both ant colonies were maintained in plastic bins (Panel Controls Corp., Detroit, MI) (41 cm long by 38 cm wide by 11.5 cm high) lined with Fluon® (BioQuip Products, Rancho Dominguez, CA). Cells used for harborages were made of plastic Petri dishes interiorly lined with dental stone (Castone, DENTSPLY International Inc., York, PA) and topped with red cellophane to reduce light intensity. The ants were provided the three major macronutrients, lipids, proteins and carbohydrates, in various food sources. Once a week, food trays were refilled with fruit, eggs, jelly, honey and dead insects, which typically comprised of cockroaches or crickets reared on site. Ants were also supplied both tap and sugar water and were maintained at approximately 25°C, 50% RH, and on a 12:12 h (L:D) cycle. Ants used in the study were picked out of the colony containers and put directly into the laboratory bioassays without sedation.

**Bed Bugs**

Bed bugs (*C. lectularius*, Harlan strain) were known to be a pyrethroid-susceptible strain. This strain was originally collected in 1973 by Dr. Harold Harlan in Fort Dix, New Jersey. In the late 2000’s, the University of Florida’s Urban Entomology Laboratory obtained the strain and has since maintained them in colony. Colonies were reared in plastic containers (6 cm high by 8 cm diameter) with an open lid design. A 7-cm hole was drilled through the lid and then affixed with 90-µm mesh to allow for feeding. Filter paper folded accordion-style was supplied for the bed bugs to use as harborage within the containers. Using an artificial feeding system (Montes et al. 2002), bed bugs were provided defibrinated rabbit blood (Hemostat, California) once a week. Colonies were maintained in a laboratory rearing room at approximately 70% RH, 25°C,
and a photoperiod of 12:12 (L:D). Bed bugs used in the study were picked out of the colony containers and put directly into the laboratory bioassays without sedation.

**House Flies**

*Musca domestica* of the “normal” strain reared by the USDA-ARS, Center for Medical, Agriculture and Entomology (CMAVE) were provided to the University of Florida as pupae. This strain had been in colony at the facility since the 1950’s and was known to be insecticide-susceptible. Larvae were reared on a diet of 13:1:6.5 ratio of mixed wheat-bran, Calf Manna (Manna Pro Products LLC, Chesterfield, MO) and water. The pupae and adults were kept under the controlled laboratory conditions of 24-28°C, 45-55% RH and a photoperiodic L:D cycle of 8:16 h. Upon emergence, adult flies were provided water and fed a dry mixture of 1-part sugar to 1-part powdered milk. For laboratory bioassays, house flies were aspirated using a handheld vacuum (Shark® Cordless Pet Perfect II Hand Vac, SharkNinja Operating LLC, Newton, MA) and placed in the freezer (-20°C) for approximately 2 minutes until immobile. Quiescent flies were placed on a chilled metal tray, which was lined with wax paper, to be sexed and counted prior to their introduction to the assay.

**Fruit Flies**

*Drosophila melanogaster* adults of the wild-type, Oregon-R strain (Carolina Biological Supply Company, Burlington, NC) were purchased in 2014 and have since been maintained in the Urban Entomology Laboratory. Colonies were contained in plastic vials (No. 55-50, Thornton Plastic Co., Salt Lake City, UT) with a sponge cap. Blue Instant Drosophila Medium (Formula 4-24®, Carolina Biological Supply Company, Burlington, NC) was used as a substrate and food source for *D. melanogaster* flies. Flies were maintained at approximately 25°C, 50% RH, and a photoperiod of 12:12 h
Drosophila repleta adults were purchased from the San Diego Species Stock Center in 2009 and have remained in colony at the Urban Entomology Laboratory. Flies were reared under the same conditions as D. melanogaster, but were instead fed Plain Instant Drosophila Medium (Formula 4-24®, Carolina Biological Supply Company, Burlington, NC). For laboratory assays, CO$_2$ was gently released for 5-7 sec into the colony vials of both Drosophila species until flies became immobile. Static flies were counted and sorted on a chilled metal tray lined with wax paper (Reynolds® Cut-Rite, Reynolds Consumer Products, Richmond, VA) prior to their introduction to the assay.

Mosquitoes

A colony of Ae. aegypti with no known resistance to insecticides was provided by the Center of Medical, Agricultural and Veterinary Entomology (CMAVE) of the USDA-ARS facility located in Gainesville, Florida. The Urban Entomology Laboratory rearing room that contains both adult and immature stages was maintained at approximately 28°C and 36% RH with a 12:12 h photoperiod (L:D). A 10% sucrose solution and a weekly blood-meal was made available to adult mosquitoes. In accordance with the regulations outlined in IACUC Protocol #201603836, mosquitoes blood-fed on the legs of a chicken, which were slipped through the cloth sleeve of the rearing cage and held there until the majority of the females reached engorgement. Strips of filter paper lined the wall of plastic 470 mL cups (WNA, Covington, KY) filled halfway with well-water. Cups containing the filter paper and water were placed in the mosquito rearing cages to allow oviposition by females. After eggs had been laid, the filter paper egg-sheets were removed and stored in Ziploc® (SC Johnson, Racine, WI) twist-top containers. A 60 mL cup of water was placed within the containers to maintain high relative humidity to preserve eggs. Egg hatch was induced by submerging egg-sheets into pans (39 cm x
51 cm x 8 cm, Del-Tec/Panel Controls, Greenville, SC) of well-water with ~2 g of ground
goldfish flakes (Tetra Fin®, Blacksburg, VA) added daily for larval diet. Pupae are
moved into plastic 470 mL cups (WNA, Covington, KY) 2/3rd filled with well-water and
placed into a rearing cage (30 x 30 x 30 cm, Bioquip®, Rancho Dominguez, CA, USA)
to allow for adult emergence. For laboratory bioassays, adult mosquitoes were drawn
from cages using a mechanical aspirator (Clarke Environmental®, St. Charles, IL, USA)
and placed into the freezer (-20°C) for approximately 2 minutes, until they were
immobile. Mosquitoes were then sexed and counted on a cold tray to maintain
immobility prior to their introduction to the assay.

Bioassays and Exposure Arenas

Two bioassays, one for crawling pest species and one for flying, were used to
expose insects to treated tile surfaces. Crawling insect species included: 1) B.
germanica 2) C. lectularius 3) C. floridanus and 4) S. invicta. Flying insect species
included: 1) D. repleta 2) D. melanogaster 3) Ae. aegypti, and 4) M. domestica.

For crawling insects, individuals were placed directly into 470 mL cups. A light
layer of talcum powder was gently sponged on the interior rim of each cup prior to
introduction to prevent insect escape. Release cups containing the insects were flipped
onto treated tiles and secured with 2 rubber bands (size 33, Office Depot, Swinton
Avenue Trading Ltd., Inc., Boca Raton, FL). After cups were flipped onto the tiles, time
of exposure was tracked and knockdown was recorded. Knockdown was characterized
by incapacitation, with the insect flipping onto its back, or curled up on its side, and
unable to recover back into an upright position.

For flying insects, individuals were allocated into laboratory-constructed release
chambers (Figure 3-1). Quiescent flying insects were placed in small Petri dishes
in the center of each Petri dish, a wooden applicator stick (8 cm x 0.2 cm diameter, Fisherbrand®) had been glued. The other piece of the release chamber was made from a 470 mL Solo® cup that had been cut 5 cm above the base. A piece of sponge (4.5 cm x 4.5 cm x 0.6 cm) was glued on the bottom of the cut cup. A hole was drilled in the middle of the base of the cup and sponge, which allowed the wooden applicator stick to be drawn through. When the applicator stick was drawn through the hole, the Petri dish was pulled to and secured against the sponge gasket on the inside of the release chamber. The Petri dish would be held against the sponge gasket until time for insect release (Figure 3-2). The assembled release chambers were secured to the tiles with 2 rubber bands and allowed for controlled release of the flying insects while preventing insect escape. Once the insects recovered from the chilled state, they were released by lowering the Petri dish with the flying insects so they could escape into the release chamber. Time of exposure was tracked and knockdown was recorded.

**Varying Concentrations of Silica and Polymer (VCSP) Formulation Study**

**Formulations: VCSP**

Four concentrations of silica were combined with four concentrations of polymer, resulting in 16 total combinations that would be evaluated. With 2 additional control formulations, a total of 18 unique formulations were prepared within 50 mL vials (Fisherbrand® 50 mL Disposable Centrifuge Tubes, Fisher Scientific, Suwanee, GA) and placed in the refrigerator (4°C) for storage after production. Concentrations of fumed silica (Cab-O-Sil®, Grade M-5, Cabot Corp., Tuscola, IL) and isobutyl-methacrylate polymer (Polysciences, Inc., Warrington, PA) used in the formulations were selected based on a preliminary study that determined what levels could be
sprayed effectively though a single-action airbrush (model H Airbrush, Paasche®, Chicago, IL, USA) without clogging (Figure 3-3). The four concentrations of silica evaluated were: 1) 0%, 2) 0.5%, 3) 1%, and 4) 1.5%. The four concentrations of polymer evaluated in this study were: 1) 0%, 2) 1%, 3) 5%, and 4) 10%.

Fumed silica and isobutyl-methacrylate polymer were measured out and allocated first for each formulation. Acetone (A18-4, Fisher Chemical, Fair Lawn, NJ) was measured and poured into the vials, which were immediately vortexed to ensure homogenization of elements into solution. Lastly, permethrin (CAS No. 52645-53-1, Lot No. B14W0321, BOC Sciences, Shirley, NY) was measured and pipetted into the vials and vortexed. The two control formulations received no permethrin. One control formulation was 100% acetone, and the other control formulation contained the greatest concentrations of silica and polymer alone in solution with acetone.

**Efficacy and Effects of Aging and Weathering on VCSP Formulations**

**Initial exposure tests**

To evaluate the 16 different combinations of silica and polymer in formulation, ceramic tiles were treated with one of the 18 formulations. After overnight storage in the refrigerator, formulations were returned to room temperature and vortexed before use. Black ceramic tiles (10.8 cm x 10.8 cm, Designer Black Tile, U.S. Ceramic Tile Co., Miami, FL) were treated at a rate of 40 mL/m² using a single-action airbrush. Under fume hood ventilation, formulations were applied to tiles at 275 kPa using gas from a compressed CO₂ tank. A total of 54 tiles were treated, with 3 replicates per treatment, per insect group.

For crawling insect species, 5 individuals were placed in 54 release cups (5 insects per cup, one cup per tile, 18 tiles [formulations] per replication, 3 replications per
treatment, for each insect species). Time started after the release cups were flipped onto the tiles, and knockdown data were collected.

For flying pest species, 5 individuals were placed in each release chamber. A total of 54 release chambers were constructed and used for each flying insect species (5 insects per chamber, one release chamber per tile, 3 tiles per treatment, for each insect species). Release chambers containing the insects were secured onto treated tiles and wooden applicator sticks were pushed down to allow the insects to fly out of the Petri dish. Once the insects escaped from the dish, the dish was drawn back up to the sponge gasket to prevent insects from reentering the dish, time started, and knockdown data were recorded.

**Aged exposure tests**

For the indoor aging study, *C. floridanus* and *Ae. aegypti* were used to evaluate efficacy of the treatments over time. The treated tiles used in the original assay for each species were retained and remained inside the Urban Entomology Laboratory at approximately 23°C for the duration of this study. Bioassays were conducted once a month for 6 months. Tiles used for *C. floridanus* were aged from July 2016 through December 2016, and the tiles used for *Ae. aegypti* were aged from August 2016 through January 2017. After each month during the aging process, the bioassays were repeated with new insects and the data were collected. For all experiments within this study, knockdown was recorded every 5 minutes for the first 30 minutes, every ten minutes for the next 150 minutes, every 30 minutes for the following 2 hours, every hour from 5 to 12 hours, and then once every 12 hours after treatment.
A replicate consisted of one treated tile for each of the 18 formulations, including controls. A total of 3 treatment replicates per month were completed for both insect species for 6 months.

**Weathered exposure tests**

The weathering study was completed by spraying 12 black ceramic tiles with each of the 18 formulations (216 tiles, 12 replicates for each formulation treatment). A replicate was comprised of one treated tile for each of the 18 formulations. *Camponotus florianus* and *Ae. aegypti* were used in the weathering study, so 6 replicates were used for each species. Three of the 6 replicates were kept indoors under normal conditions after application, and the other three replicates were exposed to weathering conditions. After weathering, the tiles were brought into the Urban Entomology Laboratory for assessment after a 24h drying period.

To expose tiles to the weathering event, they were lined up along a wooden support rack, which laid directly on the ground 100 cm from the sprinkler head. The tiles leaned against the rack at an approximately 60° angle from the ground. A simulated rain event was carried out through the use of irrigation sprinklers (Rain Bird® 1800, Rain Bird Corp., Azusa, CA) that have been calibrated to discharge 25.4 mm of water per hour. Three water collection devices (AcuRite®, Chaney Instrument Co., Lake Geneva, WI), placed 5 cm behind the rack, were positioned along the wooden rack at each end and the center of the rack (Figure 3-4). The devices were monitored to ensure that all tiles were exposed to approximately equal amounts of water. A total of 76.2 mm of water had been discharged over the tiles. After the tiles dried, insects were introduced and knockdown was recorded as previously described. Tiles that were not weathered were also tested as controls.
Varying Active Ingredient (VAI) Formulation Study

Formulations: VAI

A total of 12 formulation batches were prepared in Fisherbrand® 50 mL plastic centrifuge tubes and placed in the refrigerator (4˚C) after mixing. To compare formulations containing silica and polymer to formulations without these ingredients, half of the vials contained silica and polymer, and the other 6 did not. Concentrations of silica and polymer used in this study were selected based on results produced from the previous experiments.

Ingredients were allocated into and prepared within their respective tube as previously described. The five active ingredients evaluated in this study included: 1) permethrin, 2) fipronil, 3) indoxacarb, 4) chlorantraniliprole, and 5) chlorfenapyr. Control formulations with or without silica and polymer received no active ingredient. Appropriate concentrations of active ingredients for each formulation were derived from doses listed on pesticide labels. Amount of AI/unit area was calculated from pesticide labels for each active ingredient listed above. These rates rendered the concentrations needed to comply with the label, and were then converted to fit the standardized application rate used in this study (40 mL/m²).

Evaluation of VAI Formulation Efficacy

To assess the use of silica and polymer with different active ingredients in a residual spray solution, ceramic tiles were treated with one of the 12 formulations. After the formulations were prepared, they were kept in the refrigerator for overnight storage. Solutions were allowed to return to room temperature and were vortexed before use. Black ceramic tiles (10.8 cm x 10.8 cm, Designer Black Tile, U.S. Ceramic Tile Co., Miami, FL) were treated at a rate of 40 mL/m² using a single-action airbrush (model H
Airbrush, Paasche®, Chicago, IL, USA). Under fume hood ventilation, formulations were applied to tiles at 275 kPa using CO\textsubscript{2}. A total of 36 tiles were treated and used per insect species: 12 formulation treatments, with 3 replicates per treatment. Based on insect locomotion and feeding style/mouthparts, 4 species were selected to be used in this study: 1) \textit{C. lectularius} (crawling/piercing-sucking), 2) \textit{C. floridanus} (crawling/chewing), 3) \textit{Ae. aegypti} (flying/piercing-sucking), and 4) \textit{M. domestica} (flying/sponging). These characteristics were important to consider because the active ingredients selected for this study have different modes of entry: 1) permethrin – primarily contact, sometimes digestive, 2) fipronil – primarily digestive, sometimes contact, 3) indoxacarb – primarily digestive, 4) chlorantraniliprole – primarily digestive, and 5) chlorfenapyr – primarily contact.

For crawling insects (\textit{C. lectularius} and \textit{C. floridanus}), 5 individuals of the selected pest species were placed into 36, 470 mL Solo® cups (5 insects per cup, one cup per tile, 3 replicates per tile formulation treatment, for each insect species). Once release cups were flipped and secured onto treated tiles, time started. Knockdown was recorded for the same time intervals as previously described.

For flying insects (\textit{Ae. aegypti}, and \textit{M. domestica}), 5 individuals of the selected insect species were placed into 36 release chambers (5 insects per chamber, one release chamber per tile, 3 tile replicates per treatment, for each insect species). Release chambers containing the insects were secured onto treated tiles. Once the applicator stick was pushed down and the insects escaped the dish, sticks were drawn back up to the sponge gasket within the release chamber, time started and knockdown was recorded.
Silica and Polymer Formulations for Treatment of Bait Stations (SPFBS) Study

Formulations: SPFBS

A total of 4 formulation batches were prepared in Fisherbrand® 50 mL plastic centrifuge tubes and placed in the refrigerator (4°C) after formation. Ingredients were allocated into and combined within each tube as previously described. The active ingredient (permethrin) and concentrations of silica and polymer were selected based on the results from previous studies.

Evaluation of Silica and Polymer Formulation Efficacy in a Bait Station

To assess the novel residual spray formulation in combination with an attractive bait, 160 mL soufflé cups (Dart® Container Corp., Mason, MI) were treated with one of the 4 formulations, two contained active ingredient and two were controls: 1) permethrin + polymer and silica in acetone (A+SP), 2) polymer and silica in acetone (C+SP), 3) permethrin in acetone (A-SP), and 4) acetone only (C-SP). An “A” indicates that a formulation contained the active ingredient permethrin, and those with a “C” were control formulations, which lack the addition of permethrin. After overnight storage in the refrigerator, formulations were brought back to room temperature and vortexed before use. Soufflé cups were treated at a rate of 40 mL/m² using a single-action airbrush. Under fume hood ventilation, formulations were applied to cups at 275 kPa using CO₂.

Prior to treatment application, a 0.5 cm hole was made in the center of the base of all soufflé cups used in this study, which acted as a fly entry point. The bait dish was made by placing a 0.25 g piece of cotton in a small Petri dish (35 mm x 10 mm, Fisherbrand®, Fisher Scientific, Waltham, MA). The cotton was soaked with 3.75 g of distilled water to help prevent the bait, which would be placed on top of the saturated cotton, from drying. Based on a preliminary study, guava puree was shown to be a
highly attractive fruit source for *D. melanogaster*. Guava puree was used to make the non-toxic guava syrup bait. The syrup was made by heating a 1:1 mixture of fruit puree and sucrose in a glass beaker on a hotplate to 100°C until evenly dissolved. Six g of chilled guava syrup was apportioned onto the soaked cotton piece within the bait dish.

For the treatments receiving the bait, the filled bait dish was secured with hot glue onto the lid of the soufflé cup. Twenty-four h after treatment, soufflé cups were then inverted over and snapped onto the lid, with the entry hole on the base positioned at the top of the trap. Assembled soufflé cup traps were placed into 1.9 L plastic container arenas (GladWare® Deep Dish Containers, The Glad Products Company, Oakland, CA), along with small sugar water-filled Eppendorf Tube® plugged with a piece of cotton (Figure 3-5).

On the lids of the container arenas, holes were made and sponge gaskets were glued so that the Petri dish release devices could be drawn through and secured to as described in the previous assay (Figure 3-6). Ten *D. melanogaster* flies were placed into Petri dishes as part of the release chamber described in the previous objectives. The lids with the release devices containing the insects were secured onto the GladWare® container arenas.

The following 8 formulation/bait treatments were evaluated in this study: 1) A+SP with bait, 2) A+SP with no bait, 3) C+SP with bait, 4) C+SP with no bait, 5) A-SP with bait, 6) A-SP with no bait, 7) C-SP with bait, and 8) C-SP with no bait.

To initiate insect exposure to the treatments, the wooden applicator sticks were pushed down to allow the insects to fly out of the Petri dish. Once the insect escaped
the dish, dishes were drawn back up to the sponge gasket within the release chamber
and knockdown will be recorded. This experiment was replicated three times.

Analysis

Efficacy and Effects of Aging and Weathering on VCSP

Initial exposure tests

Time to knockdown was log transformed before analysis, which was conducted
using a 2-factor factorial ANOVA. Concentrations of silica and polymer were the main
factors in the 4 x 4 factorial experiment, and insect species was a blocking factor in the
analysis. JMP (JMP®, Pro 13 software; SAS institute 2017) was used to generate linear
regressions along the concentration gradients of silica and polymer.

Aged exposure tests

Time to knockdown (log transformed) was analyzed using a three-way ANOVA,
with insect species as the blocking factor and percent silica, percent polymer and tile
age (months) as the main effects. Means were separated using the Student’s T-test
procedure ($\alpha = 0.05$).

Weathered exposure tests

Time to knockdown (log transformed) was analyzed using a three-way ANOVA,
with insect species as the blocking factor and percent silica, percent polymer and
weather treatment (YES = rain, NO = no rain) as the main effects. Means were
separated using the Student’s T-test procedure ($\alpha = 0.05$).

Evaluation of VAI Formulation Efficacy

Insect survival over time (log transformed) was analyzed using Kaplan-Meier
survival analysis, with active ingredient and insect as the blocking factors, and the
formulation treatments (with or without silica and polymer) as the treatment groups.
Treatment groups were analyzed and means were separated using a Wilcoxon test ($\alpha = 0.05$).

**Evaluation of Silica and Polymer Formulation Efficacy in a Bait Station**

Abbott’s correction formula (Abbott 1925) was applied for the adjustment of the percent knockdown used in the analysis. The formulation efficacy within the fruit fly traps was analyzed using Kaplan-Meier survival tests, with bait treatment and formulations as the treatment groups. Treatment groups were analyzed using a Wilcoxon test ($\alpha = 0.05$).
Figure 3-1. Release chamber diagrams. Image on the right shows a Petri dish release device drawn up to the sponge gasket, preventing insect escape and allowing for controlled release.

Figure 3-2. Immobile flies are placed into the Petri dish and the release chamber is assembled and then secured onto a ceramic tile. The Petri dish device is drawn up and secured to the sponge gasket, and can be pressed down to release of the flies. (Photographs courtesy of author).
<table>
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<tr>
<td>10</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>16, 18*</td>
</tr>
</tbody>
</table>

Figure 3-3. Concentration combinations of silica and polymer in formulations 1-18. *Formulations 17 and 18 were controls that received no permethrin.

Figure 3-4. Experimental setup to weather treated tiles. (Photograph courtesy of author).
Figure 3-5. Release chamber arenas for the controlled release of *D. melanogaster*. The image on the left shows the baited trap within the container. (Photographs courtesy of author).

Figure 3-6. Release device affixed to the lid of the container assay. The left image is the lowered dish, and the image on the right shows the dish drawn up to and secured against the sponge gasket. (Photographs courtesy of author).
CHAPTER 4
RESULTS

Efficacy and Effects of Aging and Weathering on VCSP Formulations

Initial Exposure Tests

Overall in the analysis of the 8 insect species, as the amount of polymer used in formulation increased, time to knockdown increased \((F = 168.94; \text{df} = 1; p < 0.0001)\) (Figure 4-1). Additionally, the data reveal broadly that the use of silica significantly decreased the time to knockdown \((F = 22.09; \text{df} = 1; p < 0.0001)\) (Figure 4-2). Though trends were similar across the different species, the degree to which increasing concentrations of silica and polymer affected time to knockdown varied amongst insects, which can be seen in the slopes along the concentration gradient for each species \((F = 56.07; \text{df} = 7; p < 0.0001)\) (Figure 4-1, 4-2). The species that reached 100% knockdown the fastest across formulation treatments was \(B.\ germanica\), and \(C.\ lectularius\) was the slowest.

Aged Exposure Tests

Aging had a significant effect on formulation efficacy, with the 6-month aged applications having an increased time to knockdown as compared to fresh applications \((F = 32.12; \text{df} = 1; p < 0.0001)\). The percent silica in formulation had a significant effect on efficacy \((F = 3.44; \text{df} = 3; p = 0.018)\), with the highest concentration resulting in the fastest speed of kill before and after the aging period (Figure 4-3). The concentration of polymer was significant factor \((F = 154.31; \text{df} = 3; p < 0.0001)\), where formulations without polymer were more effective, both before and after the aging period, than any formulation containing polymer (Figure 4-3). The more polymer there was in the formulation, the slower the speed of kill was on fresh and aged applications. The effects
of aging, however, was not significant in the interaction with silica \((F = 1.52; df = 3; p = 0.2092)\), but was in the interaction with polymer \((F = 6.86; df = 3; p = 0.0002)\).

**Weathered Exposure Tests**

Weathering had a significant effect on formulation efficacy, with weathered applications having an increased time to knockdown as compared to fresh applications \((F = 97.37; df = 1; p < 0.0001)\). Similar to the results in the previous test, concentrations of silica had a significant effect on efficacy \((F = 13.63; df = 3; p < 0.0001)\), where formulations with the greatest amount of silica were the most effective both before and after the weathering event (Figure 4-4). Non-weathered applications were most effective, with formulations containing greater silica concentrations having the fastest knockdown times; these were the same results in the weathered treatment. Percent polymer had a significant effect on time to knockdown \((F = 171.35; df = 3; p < 0.0001)\), with lowest concentration of polymer being the most effective, regardless of weathering effects (Figure 4-4). Furthermore, these formulations containing less polymer remained more effective after the rain event than those formulations with greater concentrations of polymer that had not yet experienced any weathering. For the interactions, percent silica and rain event was not significant \((F = 1.03; df = 3; p = 0.3786)\), but the polymer x rain event interaction was \((F = 5.89; df = 3; p = 0.0007)\).

**Evaluation of VAI Formulation Efficacy**

The significance of the incorporation of silica and polymer varied among the insects, depending on the active ingredient used.

For permethrin, the formulation with silica and polymer (S&P) resulted in a significantly greater time to knockdown than the formulation without (No S&P) for each of the four insects used in the study: *C. lectularius* \((\chi^2 = 12.85; df = 1; p = 0.0003)\), *C.*
camponotus ($\chi^2 = 14.42; \text{df} = 1; p = 0.0001$), M. domestica ($\chi^2 = 24.18; \text{df} = 1; p < 0.0001$), and Ae. aegypti ($\chi^2 = 19.44; \text{df} = 1; p < 0.0001$). The formulation with permethrin in the absence of silica and polymer resulted in the fastest time to knockdown, in all cases (Figure 4-5).

For fipronil, the use of silica and polymer in formulation was only significant when tested against C. lectularius ($\chi^2 = 23.20; \text{df} = 1; p < 0.0001$) and Ae. aegypti ($\chi^2 = 16.33; \text{df} = 1; p < 0.0001$). In both cases, the formulation with fipronil alone resulted in the fastest time to knockdown (Figure 4-6).

For indoxacarb, formulation treatment was significant when tested against C. lectularius ($\chi^2 = 14.41; \text{df} = 1; p = 0.0001$) and Ae. aegypti ($\chi^2 = 5.00; \text{df} = 1; p = 0.0254$). In both cases, greatest knockdown occurred in the silica and polymer formulation treatments. Though it was not significant in the C. floridanus and M. domestica assays, the silica and polymer formulation resulted in the fastest knockdown for these species too (Figure 4-7).

For chlorantraniliprole, treatment was only significant when tested against C. floridanus ($\chi^2 = 7.40; \text{df} = 1; p = 0.0065$) and M. domestica ($\chi^2 = 9.52; \text{df} = 1; p = 0.0020$). Greatest knockdown occurred on the silica/polymer treatments for both species. This was the same outcome for C. lectularius and Ae. aegypti, however, according to the analysis, it was not significantly better than the knockdown that was produced with the formulation void of silica and polymer (Figure 4-8).

For chlorfenapyr, the formulation containing silica and polymer was significantly different than the formulation with AI alone when tested against C. floridanus ($\chi^2 = 5.44; \text{df} = 1; p = 0.0197$) and M. domestica ($\chi^2 = 27.02; \text{df} = 1; p < 0.0001$). For both species,
the formulation with silica and polymer resulted in the fastest time to knockdown (Figure 4-9).

In the control treatments, which contained no addition of AI, the incorporation of silica and polymer in formulation was only significant when tested against *C. lectularius* ($\chi^2 = 11.41; \text{df} = 1; p = 0.0007$); in both cases, the use of these ingredients resulted in greater knockdown. Knockdown was observed to only occur on the treatment containing silica and polymer (Figure 4-10).

**Evaluation of Silica and Polymer Formulation Efficacy in a Bait Station**

There was no significant difference in the percent knockdown between the formulation treatment groups; traps treated with the silica and polymer formulation were equally effective as the permethrin alone formulation (Figure 4-11). Knockdown was significantly higher in treatments that contained the attractive guava syrup bait, regardless of the formulation used: silica and polymer formulation ($\chi^2 = 6.1656; \text{df} = 1; p = 0.0130$), permethrin alone formulation ($\chi^2 = 5.4441; \text{df} = 1; p = 0.0196$) (Figure 4-12). Knockdown in the control-baited treatments was a result of flies getting stuck in the syrup bait. Across replication, 33% knockdown occurred in these treatments, and was adjusted for using Abbot’s correction.
Figure 4-1. Time to knockdown (log transformed) for A) flying and B) crawling insect species as concentrations of polymer increased in VCSP formulations.
Figure 4-2. Time to knockdown (log transformed) for A) flying and B) crawling insect species as concentrations of silica increased in VCSP formulations.
Figure 4-3. Time to knockdown (log transformed) for *C. floridanus* and *Ae. aegypti* on fresh (0 months) and aged (6 months) applications as concentrations of A) polymer and B) silica increased in formulation.
Figure 4-4. Time to knockdown (log transformed) for *C. floridanus* and *Ae. aegypti* on fresh (NO rain) and weathered (YES rain) applications as concentrations of A) polymer and B) silica increased in formulation.
Figure 4-5. Percent survival of A) *C. lectularius*, B) *C. floridanus*, C) *M. domestica*, and D) *Ae. aegypti* over time (log transformed) on permethrin formulations that contained silica and polymer (S&P), and that did not contain silica and polymer (No S&P).
Figure 4-6. Percent survival of A) *C. lectularius*, B) *C. floridanus*, C) *M. domestica*, and D) *Ae. aegypti* over time (log transformed) on fipronil formulations that contained silica and polymer (S&P), and that did not contain silica and polymer (No S&P).
Figure 4-7. Percent survival of A) *C. lectularius*, B) *C. floridanus*, C) *M. domestica*, and D) *Ae. aegypti* over time (log transformed) on indoxacarb formulations that contained silica and polymer (S&P), and that did not contain silica and polymer (No S&P).
Figure 4-8. Percent survival of A) *C. lectularius*, B) *C. floridanus*, C) *M. domestica*, and D) *Ae. aegypti* over time (log transformed) on chlorantraniliprole formulations that contained silica and polymer (S&P), and that did not contain silica and polymer (No S&P).
Figure 4-9. Percent survival of A) C. lectularius, B) C. floridanus, C) M. domestica, and D) Ae. aegypti over time (log transformed) on chlorfenapyr formulations that contained silica and polymer (S&P), and that did not contain silica and polymer (No S&P).
Figure 4-10. Percent survival of A) *C. lectularius*, B) *C. floridanus*, C) *M. domestica*, and D) *Ae. aegypti* over time (log transformed) on formulations with no pesticidal active ingredients that contained silica and polymer (S&P), and that did not contain silica and polymer (No S&P; acetone alone).
Figure 4-11. Percent knockdown of *D. melanogaster* over time (log transformed) when exposed to baited and non-baited traps that were treated with permethrin formulations that contained silica and polymer (S&P), and that did not contain silica and polymer (No S&P).

Figure 4-12. The Kaplan-Meier survival plot, representing the percent survival of *D. melanogaster* over time (log transformed) when exposed to different bait and formulation treatment combinations. Formulations either contained silica (S) and polymer (P), or did not.
CHAPTER 5
DISCUSSION

Our findings indicate that formulations containing greater concentrations of isobutyl-methacrylate polymer were less effective in maintaining the immediate and residual activity of the application. Lower concentrations of polymer resulted in faster knockdown times, with formulations containing no polymer providing the fastest knockdown on both fresh and aged applications. Previous studies evaluating the efficacy of polymer-enhanced (PE) formulations have shown dissimilar results on how successful the use of polymers is in improving or maintaining the insecticidal activity of an application. A study by Brook et al. (2014) found that there was not a significant decrease in mortality of *Anopheles arabiensis* on surfaces sprayed with a deltamethrin SC-PE (Suspension Concentrate – Polymer Enhanced) formulation 12 months post-application. Additionally, the PE formulation in the Brook et al. study resulted in significantly higher overall mortality when compared to the other formulation types evaluated. However, the present study shows that there was a decrease in efficacy after 6 months in formulations containing polymer, with greater concentrations being the least effective over time. Discrepancies could potentially be a result of the use of different polymer types, or formulation preparation.

In the current study, the addition of greater concentrations of polymer, in general, affected the knockdown times similarly across the insect species tested. The use of polymers in an insecticide is described as a way to allow active constituents to slowly and continuously diffuse from the matrix (Mogul et al. 1996). Therefore, it can be expected that greater amounts of polymeric material would affect the discharge rate of the insecticidal chemical. Another study evaluating two residual insecticide products,
Fendona® and Suspend Polyzone®, was conducted to determine immediate and long-term efficacy to control nuisance ants (Basnet et al. 2015). Fendona® remained equally effective as the Suspend Polyzone®, which is a polymer formulation, throughout the course of the study. Unlike the Brooke et al. study, the formulations used in Basnet’s study showed the significant negative effects that age had on application efficacy, which is more comparable to the results obtained in this present study. Polyzone® was also evaluated by the USDA in a study aimed to control house flies and stable flies (Hogsette 2014). After pieces of cloth were treated with a label-rate application and exposed to outdoor weathering conditions, flies were briefly exposed to the material. Results produced showed that residuals on non-weathered treatments were better than the residuals on the cloths that had been subjected to outdoor weathering. But regardless of treatment, all flies tested were dead within 24h post-exposure to the material. Formulations that contained greater concentrations of polymer in the present study significantly lost efficacy after exposure to the artificial rain event. When using a polymer-based formulation in areas exposed to these conditions, it is necessary to consider the surface material upon which the application is being made. The inability to effectively adhere to the surface of application has potential consequences, such as reduced residual. The beneficial qualities of Suspend Polyzone® have been identified and claimed, including its long-lasting formulation that resists erosion and protects the AI from abrasion and weather. The acceptance of these properties has resulted in recommendations for its use in vector control of the yellow fever mosquito (Woodall 2016). However, polymers used in the current study, which are likely similar to that of
the one used in Polyzone®, were not effective in protecting the active ingredient from the negative effects of weather or age.

Our findings indicate that the use of greater concentrations of silica improved the immediate effectiveness of an application and assisted in the preservation of the residual activity when applications were subjected to the effects of aging and weathering. Bartlett (1953) evaluated the effects field-weathering without rainfall on dust formulations, finding that weathering significantly decreased the efficacy of the treatment, with time beyond 1 week of weathering requiring over 100 hours of exposure to result in LT50. The addition of silica dust in the formulations evaluated in our study, however, showed to improve the residual effectiveness when under conditions of degradation, including maintaining the insecticidal activity of a 6-month old application. The addition of this sorptive-dust material to a spray formulation containing an accompanying AI has improved the overall efficacy initially, over time, and upon weathering.

The current study demonstrates the capacity for improving the immediate and residual effectiveness of various active ingredients when formulated with silica and polymer. In formulation, silica and polymer operate by absorbing the AI, but the modes in which the chemicals are retained and released over time within these materials differ. As expected, for the formulation treatments evaluated with firpronil, indoxacarb, chlorantraniliprole, and chlorfenapyr, the degree of difference between the formulations with and without the addition of silica and polymer varied depending on the insect species the applications were being tested against. Though results varied among the insect species used, formulations that contained both materials improved the
knockdown time when combined with chlorfenapyr, chlorantraniliprole and indoxacarb. Studies that would look at the effectiveness of these formulations over time and after weathering would be helpful to understand the additional potential benefits associated with these ingredients when used in combination with the listed AIs. The results generated from using silica and polymer with other active ingredients warrant further study.

Our study indicates that the use of an attractive bait significantly improved the efficacy of treated traps, regardless of the use of silica and polymer (S&P) in trap treatment. This is an important finding because, unlike in the forced exposure tests, there was no significant decrease in knockdown time for the S&P formulation; both formulations types were equally effective, which indicates that there is no repellent factor associated with the use of these materials. Similar results have been reported, showing that the use of bait will significantly improve trap catch (Zhu et al. 2003). In the study conducted by Zhu et al. (2003), overripe mango was the most attractive to D. melanogaster; though guava was not tested, it does share similar volatile compounds with mango. With the results obtained from our previous studies, it can be determined that traps containing silica + AI would provide an effective residual for treatment of this type of fruit fly trap.
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BIOGRAPHICAL SKETCH

Heather A. Erskine was born and raised in Lakeland, Florida to Dean and Patti Erskine. She has two sisters, Julie Johnson and Lindsey Erskine, who both work as teachers in Lakeland. Heather grew up on the Reservation Golf Course; an 18-hole course her grandfather built in Mulberry, FL in 1969. At the age of 12, she began working summers out there, and continued to work at the Reservation throughout her young adult life prior to moving to Gainesville to attend the University of Florida in 2013. During high school, Heather was heavily involved in Future Farmers of America (FFA); her participation allowed her to raise livestock for show, assume leadership positions, and investigate career possibilities within agriculture. Her outdoor work and involvement in FFA ignited and shaped her interests in entomology – she graduated Cum Laude with her Bachelor of Science degree in entomology and nematology in 2015 from UF. While acquiring her BS, she worked in several laboratories in the Department of Entomology, one of which focused on urban pest management. Here Heather conducted bed bug research, and was able to continue onto an MS program working under Dr. Phil Koehler and Dr. Roberto Pereira. She graduated with her degree in summer of 2017, and her research focused on evaluating the use of silica and polymer within a residual insecticide formulation against major pest insects. Upon graduating with her MS degree in entomology, Heather began working in the Pest Management Industry. She is an ardent entomologist and looks forward to growing herself as a leader and working member of this field.