IMPACT OF THE INSECT GROWTH REGULATOR PYRIPROXYFEN ON LIFE TABLE CHARACTERISTICS OF *AEDES ALBOPICTUS*

by

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Aedes albopictus is a vector of several arboviruses, including dengue, chikungunya, and Zika. However, control of this day-active species is difficult with ultra-low volume treatments applied at dusk/dawn periods. Consequently, the impacts of an alternative method (residual barrier spray) used to control resting mosquito adults were evaluated. Eggs were collected from field study sites treated with Demand CS® (pyrethroid adulticide) (active ingredient [AI]: lambda-cyhalothrin) plus Archer® (insect growth regulator larvicide) (AI: pyriproxyfen) at pre-determined concentrations and life table characteristics assessed in the laboratory. In a separate laboratory study, blood fed *Ae. albopictus* were exposed to Archer® residue in glass bottles (to approximate contact from a barrier spray) and subsequently allowed to oviposit. Control mosquitoes were exposed to clean bottles. Mosquitoes were held in incubators at 28°C for the duration of the experiments. To evaluate potential dilution effects of water volume, mosquitoes were allowed to oviposit in (relatively) small (59 mL water) or large (177 mL water) containers. We characterized the extent to which fecundity (number of eggs laid), fertility rate (number of larvae hatched/number of eggs laid*100), and emergence rate (number of adults emerged/number of larvae hatched*100) differed between groups. In the control group, 18-21 (82-95%) mosquitoes laid eggs, while only 10-11 (45-50%) mosquitoes laid eggs in the group exposed to pyriproxyfen. Significantly lower

(*P*=0.0008) fecundity was observed in mosquitoes exposed to pyriproxyfen (mean±SE) (small container: 25.2±7.1, large container: 24.3±7.1) compared to control mosquitoes (small container: 49.2±7.8, large container: 52.7±5.2). Regardless of treatment, no significant differences in fecundity were observed between mosquitoes allowed to oviposit in different sized containers. Hatch rate was significantly lower in the pyriproxyfen group and was impacted by size of container (*P*=0.032) and treatment (*P*< 0.0001) (large, control: 61.9% ± 7.8; small, control: 38.0% ± 7.1; large, treated: 10.3% ± 2.4; small, treated: 2.9% ± 1.9). Adult emergence rates were not significantly impacted by treatment or size of container. Pyriproxyfen applied as a barrier spray may be an effective tool for controlling *Ae. albopictus* and other peridomestic mosquitoes.

Impact of the insect growth regulator pyriproxyfen on life table characteristics of *Aedes albopictus*

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CHAPTER I - INTRODUCTION AND PURPOSE OF THE STUDY

Increased insecticide resistance, precipitation, humidity, and elevated temperatures generally create more favorable conditions for mosquitoes. Therefore, control methods should be evaluated to improve efficiency and provide the most efficacious mosquito control. *Aedes albopictus* (Skuse) is an invasive mosquito species and a competent vector of dengue virus (DENV), chikungunya virus (CHIKV), Zika virus (ZIKV) and other arboviruses of public health concern. In the absence of vaccines, reduction of oviposition sites and vector control is the only means of controlling these diseases (Chandel et al. 2016). Routine source reduction/removal of water-holding containers (e.g., tires, buckets, tarps) can help reduce populations of container-ovipositing mosquitoes, such as *Ae. albopictus*.

Larvicides can be applied to mosquito oviposition sites to control mosquitoes before they emerge as adults. Larval oviposition sites for *Ae. albopictus* are diverse, ranging from natural sites (e.g., bamboo stumps, tree holes, bromeliads) to artificial containers (e.g., discarded tires, soda cans, and water storage containers including plant pots, bird baths, and drainage pipes (Hawley, 1988). Gravid *Ae. albopictus* females may prefer cryptic cups over open containers for oviposition (Chandel et al. 2016). However, the potential of ground-based larvicide applications to reach hidden or cryptic larval environments is not well established for container ovipositing species (Chandel et al. 2016). Control of mosquito larvae can be used as part of an integrated pest management (IPM) approach; however, this may be difficult to implement over large urban and/or suburban areas (Fonseca et al. 2013). Adulticides can also be used

in some cases; however, research should investigate methods that inhibit growth and reproduction cycles before mosquitoes emerge as adults.

Aedes albopictus is abundant in artificial containers such as tire habitats, where larval development takes place (Qualls & Mullen 2006, Yee 2008, Yee et al. 2012). The rapid global spread of *Ae. albopictus* is a public health concern due to its vector competence for at least 20 arboviruses (Paupy et al. 2009). This nuisance species and its opportunistic blood feeding habits (including humans) lead to frequent biting during the day that is a significant deterrent of outdoor recreation (Dowling et al. 2013).

Pyriproxyfen is an insect growth regulator (IGR) that mimics natural insect hormones to stop young insects from maturing into adults; however, it is thought that pyriproxyfen is rarely toxic to adult insects (Hallman et al. 2015). Products containing pyriproxyfen can be mixed with adulticides to ensure adult insects are killed (Hallman et al. 2015). For example, the adulticide Demand CS (AI: lambda-cyhalothrin) has been combined with the IGR larvicide Archer (AI: pyriproxyfen) to control pest populations at all life stages. Pyriproxyfen affects many types of insects, including fleas, cockroaches, ticks, ants, carpet beetles, and mosquitoes (Hallman et al. 2015)

Lambda-cyhalothrin belongs to a group of chemicals called pyrethroids. Scientists developed pyrethroid (manmade chemicals) insecticides to have properties like those of the pyrethrins (natural insecticides) (WHO 1990). Pyrethroids, including lambda-cyhalothrin, disrupt the normal functioning of the nervous system in invertebrate organisms; therefore, exposure to lambda-cyhalothrin may cause paralysis and/or death in insects (WHO 1990). Lambda-cyhalothrin has been used primarily for controlling

pests such as mosquitoes, fleas, cockroaches, flies, and ants around households (Zhao et al. 2008).

In a previous study involving *Ae. albopictus,* the efficacy of pyriproxyfen autodissemination stations was assessed in cryptic and open plastic cups (containing 250 mL of tap water) in residential areas (Chandel et al. 2016). Autodissemination stations attract gravid female mosquitoes searching for oviposition sources and subsequently contaminates mosquitoes with pyriproxyfen (Gaugler et al. 2012). As female mosquitoes exit the autodissemination station, they may transfer the pyriproxyfen to other oviposition sites, thereby providing control in multiple oviposition sites (Gaugler et al. 2012). A study showed that a powder formulation of pyriproxyfen-impregnated silica particles adhered to *Ae. albopictus* females visiting autodissemination stations (Gaugler et al. 2012). The station consists of a water reservoir to attract gravid females, which is joined to a transfer chamber that contaminates mosquitoes with pyriproxyfen (Gaugler et al. 2012). In a separate study, oviposition preference was determined by counting the number of cups that received eggs and the number of eggs that had accumulated within each cup (Chandel et al. 2016). The same study showed that pyriproxyfen effectively contaminated cryptic cups (59 - 85%) and produced 30 - 41% pupal mortality (Chandel et al. 2016). *Aedes albopictus* females deposited 84% of their eggs in cryptic cups; however, open cups only received 16% of eggs (Chandel et al. 2016).

The Division of Vector Borne Diseases suggests science-based guidance along with nationwide disease surveillance to combat arboviruses of public health concern such as WNV, DENV, CHIKV, JEV, and yellow fever virus (YFV) (CDC, 2013). Many

mosquito control programs simply do not possess the man-power and financial resources to suppress *Ae. albopictus* effectively in peridomestic environments (Faraji and Unlu, 2016). In many cases, the public may turn to private pest management companies for assistance with mosquito control (e.g., barrier sprays) in their yards. Therefore, it is vital that the efficacy of barrier spray products be evaluated.

The central hypothesis of the current study is that pyriproxyfen impacts fecundity, fertility, and eclosion in *Ae. albopictus*. Although previous research has examined variation in oviposition behavior affected by IGRs, little work has evaluated impacts on life table characteristics for this AI in *Ae. albopictus.* Here, we evaluate the effectiveness of a product used in barrier sprays for mosquito control (Demand CS: AI lambda-cyhalothrin with Archer: AI pyriproxyfen]).

Study Objectives:

1. Evaluate the extent to which the barrier spray Demand CS (AI: lambda-cyhalothrin) with Archer (AI: pyriproxyfen) impacts life table characteristics (fecundity, fertility, eclosion rates) of *Ae. albopictus* in a suburban field environment.
2. Characterize the extent to which Archer (AI: pyriproxyfen) impacts life table characteristics (fecundity, fertility, eclosion rates) of *Ae. albopictus* under laboratory conditions.

CHAPTER II - LITERATURE REVIEW

Introduction

Humans' increased mobility, international trade, and increasing temperatures support the spread of mosquitoes and play an important role in the dissemination of the vectors and their pathogens/parasites (Becker 2008). Climate change may influence *Ae. albopictus* by creating conditions that increase vector abundance and transmission of pathogens that cause disease (Little et al. 2017). *Aedes albopictus* is a major public health problem due to its ability to transmit DENV, CHIKV, yellow fever virus (YFV), and is a competent vector for at least 22 other arboviruses including ZIKV (Gratz, 2004). There is the potential for an exotic pathogen outbreak by *Ae. albopictus* and mosquito control is the most effective method of reducing transmission risk to humans during epidemics. Hence, it is imperative that effective control strategies are developed and implemented to protect public health (Faraji and Unlu 2016).

Life table characteristics of *Aedes albopictus*

Aedes albopictus adapts to the environment via physiological and ecological plasticity and this has contributed to the global growth of this invasive, capable, vector species (Paupy et al. 2009). Vector population growth is directly influenced by ecological processes, including climatic conditions and resource quality, during immature stages (e.g., eggs, larvae, and pupae) (Kraus and Vonesh 2012, LaDeau et al. 2015). Precipitation and temperature directly impact *Ae. albopictus* populations. Precipitation allows oviposition sites to fill up with water to provide an environment supportive of larval and pupal development. Furthermore, temperature can have both

direct and indirect influences on immature and adult mosquito survival, development, and adult female blood feeding behaviors (Alto and Juliano 2001).

Elevated environmental temperature may result in increased probability of mosquito survival to adulthood and rapid larval growth and development (Teng and Apperson 2000). However, in these cases, mosquito adults may be smaller, with correspondingly reduced fecundity and reduced longevity (Rueda et al. 1990, Hawley 1985, Day et al. 1990). *Aedes albopictus* eggs can survive extreme weather conditions (in microhabitats that buffer these conditions) and diapausing (dormant) eggs can survive drought and winter conditions in some geographic regions (Becker et al. 2012). A previous study evaluating the environmental suitability for *Ae. albopictus* in the US reports that *Ae. albopictus* can survive in temperatures ranging from -5 to 40.6 °C (Gao et al. 1984). The plasticity of the genome enables these mosquitoes to adjust to different environmental and ecological conditions (Becker et al. 2012). The ability of *Ae. albopictus* to resist cold temperatures is likely linked to its ability to synthesize a high amount of lipids and to product larger amounts of yolk lipid in cold temperatures (Paupy et al. 2009).

Ae. albopictus, a highly urbanized container-dwelling species, colonizes cryptic larval habitats, and is a skip ovipositor that distributes eggs among multiple water-holding containers (Trexler et al. 1998). This species tends to opportunistically select oviposition sites and utilizes a broad range of container types and sizes, including small ephemeral containers that may harbor small populations of this invasive species (Becker et al. 2014, Richards et al. 2008, Barlett-Healy et al. 2012). The occurrence of water-holding containers may change on a continual basis due to people moving,

removing, and/or adding containers, as well as changes in weather patterns. This makes source reduction and control of this species even more difficult (Fonseca et al. 2013, Faraji et al. 2014, Unlu et al. 2015). Shifts in biological or ecological behaviors, such as habitat preference and skip oviposition, continue to further confound control efforts for *Ae. albopictus* (Faraji and Unlu 2016).

Habitat and climate influence on reproduction

Habitat:

The habitat of *Ae. albopictus* is likely influenced by local conditions that support larval development, resting survivorship, and host access within its 100 m flight range (Marini et al. 2010). *Aedes albopictus* originated in the forests of Southeast Asia, where it was likely zoophilic (blood feeding on wildlife); however, the species progressively adapted to anthropogenic environmental changes, which provided alternative blood sources (domestic animals and humans) and water for larval habitats (Paupy et al. 2009). Unlike mosquitoes that thrive in flood water and other habitats where stagnant water is present, *Ae. albopictus* larvae flourish in small pockets of water in natural and artificial containers that are often cryptic, ubiquitous, and widely distributed within peridomestic environments (Unlu et al. 2013, Unlu et al. 2014a). Cryptic containers (e.g., cisterns and/or pots, cans, buckets under heavy vegetative cover) make eliminating larval sites difficult using conventional methods.

Aedes albopictus adults usually rest in dense vegetation surrounding oviposition sites, and human habitations such as brushwood (Chun et al. 2010). High *Ae. albopictus* population densities, overwhelming and recurring amounts of larval habitats, and a large variety and inaccessibility of resting sites that may be protected from

treatments may reduce the effectiveness of adulticide applications (Faraji and Unlu 2016). It is clear that ecological and environmental variability throughout the invasive range of *Ae. albopictus* must be considered for residual applications to be effective (Faraji and Unlu 2016). Hence, barrier or residual applications against *Ae. albopictus* should concentrate on focal areas that may support large larval populations and/or selected resting sites for peridomestic adult mosquitoes (Faraji and Unlu 2016). If access and labor-time issues are of primary concern, larger residual applications may be conducted in public areas such as parks, gardens, or golf courses, where human activity may be high and sufficient vegetation and humidity provide adequate resting sites for adult mosquitoes (Faraji and Unlu 2016).

Impacts of climate on Ae. albopictus:

Globally rising temperatures, along with increasing events of heavy precipitation, facilitate the introduction and establishment of mosquito populations, as well as vector-borne pathogens (Becker 2008). Predicted climate changes are likely to cause a northward shift in the current distribution of *Ae. albopictus* by decreasing winter mortality due to a decrease in the number of winter days with extremely low temperatures (Focks et al. 1994, Hanson and Craig 1995). Warmer summer temperatures may also contribute to the northern expansion of *Ae. albopictus* (Alto and Juliano 2001).

In the absence of human mediated water sources, precipitation is necessary for egg deposition (Medlock et al. 2015). Precipitation facilitates growth of mosquito populations by filling containers and other water sources to provide sites for oviposition and juvenile development (Alto & Juliano 2001, Unlu et al. 2014). Warmer temperate regions are likely to have greater production of *Ae. albopictus* adults as long as

container habitats do not dry completely (Bradshaw and Holzapfel 1984). Increasing frequency of habitat drying would result in lower numbers of adults (Alto and Juliano 2001). In contrast, populations in cooler, wetter temperate regions are likely to produce fewer adult *Ae. albopictus*, and variation in precipitation contributes less to variation in production of adults than temperature (Alto and Juliano 2001).

Blood feeding patterns of *Aedes albopictus*

Aedes albopictus is mainly a daytime and exophagic mosquito, preferring to blood feed during the day and late afternoon. However, exceptions to this have been recorded and depend on the season, region, host availability and the nature of the habitat (Paupy et al. 2009). Mosquitoes may become infected with ZIKV, DENV, or CHIKV when they blood feed on an infectious person (during the viremic phase of infection) (CDC 2016). Vector competent mosquitoes can potentially transmit pathogens that cause disease to susceptible hosts, by injecting infectious saliva during a subsequent blood meal (CDC 2016). *Aedes albopictus* are opportunistic blood feeders, primarily feeding on mammals, but also blood feed on other types of hosts (e.g., reptiles, birds, and amphibians) (Scholte and Schaffnar 2007). Mosquito blood feeding patterns are a critical component of potential virus proliferation in enzootic and epidemic transmission cycles and determine, in part, the degree and intensity of disease epidemics (Faraji et al. 2014). The propensity of *Ae. albopictus* to blood feed on different types of vertebrates may impact biological traits (e.g., fecundity and survival) and disease risk (Paupy et al. 2009). Vectors' ability to successfully transmit pathogens that cause disease is related to the range of hosts on which they feed and environmental factors.

Geographic spread

Increased international travel of humans and range expansion of mosquitoes due to global warming has, in part, resulted in the range expansion of some mosquito-borne pathogens to locations where they have previously been eradicated or were nonexistent (Becker 2015, Yee et al. 2014, Lounibos 2002). Knowledge of distribution patterns, temporal abundance, and habitat preferences of potential vectors allow public health officials to more accurately predict the location and timing of potential outbreak events (Champion & Vitek 2014). *Aedes albopictus,* originally a zoophilic forest species from Asia, rapidly expanded its range to Europe, the US, and Brazil in the 1980s (Medlock et al., 2012). *Aedes albopictus* was introduced into the Americas in the 1980s through imported tires and bamboo plants, and it has since spread rapidly (Bonizzoni et al. 2013). The first established population of *Ae. albopictus* in the continental US was recorded from Texas in 1985 (Sprenger and Wuithiranyagool 1986). The mosquito thereafter spread rapidly across the Southeast to later reach the southern portions of the Northeast and Upper Midwest as well as the Pacific Coast (Kraemer et al. 2015). Today, *Ae. albopictus* can be found in a number of countries (ranging from the US to Argentina), numerous Pacific Islands (e.g., Hawaii, the Solomon Islands and Fiji) and in Australia. In Africa, *Ae. albopictus* was first detected in 1989 in South Africa and later in Nigeria, Cameroon, Equatorial Guinea and Gabon (Paupy et al. 2009).

Ecological, demographic, behavioral, and genetic studies indicate that *Ae. albopictus* can tolerate climate/environment interactions that differ from its native range (Gusian et al. 2014, Costanzo et al. 2015, Brady et al. 2014). This mosquito species has been increasingly involved in local autochthonous transmission of CHIK and DENV in

many places where it has become established, including La Reunion (France), continental Europe, Africa, The Americas, and Japan (Morrison 2014, Suter et al. 2016). In Europe, the first CHIKV outbreak took place in 2007 in Italy with more than 200 confirmed cases (Carrieri et al. 2012). The first report of *Ae. albopictus* in Europe was in Albania in 1979, and this species has since been detected in other European countries such as Bosnia and Herzegovina, Croatia, Greece, France, Italy, Montenegro, the Netherlands, Serbia, Sloveniz, Spain, and Switzerland (Scholte and Schaffner 2007).

Due to the ability of *Ae. albopictus* to colonize a wide range of natural and artificial oviposition sites, the resistance of its eggs to desiccation and cold (via diapause), and its opportunistic blood feeding, this species has been able to rapidly build populations in a variety of geographic regions (Becker 2008). This species is predicted to continue to expand its geographical range in the coming years as a result of urbanization, habitat suitability, transportation of eggs/larvae in artificial containers, and global climate change (Rochlin et al. 2013, Ogden et al. 2014). The establishment of this species into new areas, particularly highly dense human population centers, will strain the resources of mosquito control programs and increase the public health threat for arboviruses such as CHIKV, DENV, and ZIKV (Gratz 2004, Faraji et al. 2014).

Capacity of *Ae. albopictus* to transmit pathogens that cause disease

After examining blood meal sources of *Ae. albopictus* at their northernmost locations in North America, it was found that the species fed primarily on mammalian hosts, with over 90% of blood meals derived from humans and their associated pets (Faraji et al. 2014). The same study suggests that the high mammalian affinity of *Ae. albopictus* may lead it to be an efficient vector of mammal-driven zoonoses and human-

driven anthroponoses such as DENV and CHIKV in this region (Faraji et al. 2014). Others have shown an association between host availability and blood feeding preference in *Ae. albopictus* (Richards et al. 2006). Zika virus, commonly associated with Guillian-Barre syndrome in adults, has recently shown to increase the risk of microcephaly in fetuses of infected pregnant women (Kostyuchenko et al. 2016). *Aedes albopictus* are increasing in abundance within metropolitan centers and thrive in artificial containers found in close association with peridomestic environments (Faraji et al. 2014). This, combined with the emergence and resurgence of exotic pathogens for which *Ae. albopictus* is a competent vector, show that it is essential to investigate this species further to understand its role in disease ecology and public health significance (Faraji et al. 2014).

Integrated Mosquito Management

Integrated Mosquito Management (IMM) programs are built upon the foundation of physical, biological, and chemical control methods which are supported by community participation and involvement (Becker 2008). Physical control measures include environmental management of oviposition sites through source reduction and community education. Some examples include cleaning roof gutters and avoiding collection of water-holding containers (Becker et al. 2012). Biological control measures to control container ovipositing mosquitoes are mainly based on microbial control agents, IGRs, and/or natural predators (Becker 2015).

When considering IMM, the treatment method(s) selected should be efficient, effective, economically sound, and reduce environmental impacts (Faraji & Unlu 2016). Over hundreds of million years, mosquitoes have evolved and survived in many

different natural and artificial aquatic habitats (Becker et al. 2015). The control of container-inhabiting mosquitoes is based on environmental management, with special emphasis on community participation (e.g., source reduction by elimination or drainage of areas with standing water). However, citizens are often not aware of the problems related to urban mosquito control and/or ignore advice provided during anti-mosquito control campaigns (Becker et al. 2015).

Source reduction, adulticides, and larvicide applications are routinely used to manage *Ae. albopictus* (Faraji & Unlu 2016). Eliminating the diverse array of containers used by peridomestic *Ae. albopictus* is extremely challenging and labor intensive. While adulticides may be effective for suppressing adult mosquito populations, adult populations may rebound due to sub-lethal chemical exposure contributing to insecticide resistance (Faraji & Unlu 2016, Fonseca et al. 2013). Vector control methods may include larval source reduction or the use of ultra-low volume (ULV) insecticides applied at dusk or dawn. However, ULV applications are not effective for day-active *Aedes* and some mosquito populations have developed resistance to many commonly used insecticides (Bartlett-Healy et al. 2012, Leisnham and Juliano 2012, Marcombe et al. 2014).

Adulticides and larvicides for mosquito control

Because of the challenges in controlling container-inhabiting mosquitoes within residential areas, researchers have investigated the use of area-wide larviciding. Similar to ULV delivery of adulticides, liquid larvicides can be delivered using blowers; however, the major difference between the two approaches is the size of the droplets produced with each method (Faraji and Unlu, 2016). For ULV adulticiding, a droplet size range of

5-25 μm is the most efficient, because this size is most likely to stay adrift and come into contact with adult flying mosquitoes (Haile et al. 1982, Bonds 2012). For larviciding, a larger droplet size (100-300 μm) is required so that droplets can stay aloft temporarily, but ultimately settle into containers holding water (Williams et al. 2014). An entire neighborhood can be treated in one night with truck-mounted larvicide application and does not require homeowner permission to enter the property.

Autodissemination stations, attractive sugar baits, and genetic control

Autodissemination uses insects contaminated with a biological or chemical insecticide to transfer lethal concentrations horizontally or vertically to other insects via mating, oviposition, aggregation and other behaviors (Gaugler et al. 2012). Autodissemination is a “pull” (attraction and transfer) and “push” (dispersal and transfer to target habitats) technology (Gaugler et al. 2012). This targeted approach offers the potential for economic (savings in product and labor) and environmental (lower amount of AI) benefits relative to broadcast spray applications (Gaugler et al. 2012). Another component of the autodissemination approach has been the exploitation of male mosquitoes to transfer pyriproxyfen (an IGR) either directly to larval habitats or indirectly through sexual contact to females during mating. Sexual transmission of pyriproxyfen from contaminated males to virgin females has been recorded in the laboratory (Gaugler et al. 2012). Pyriproxyfen (dust) has been observed clinging to various body regions, including the tarsi, and were often found attached to the adult female’s last two abdominal segments (Gaugler et al. 2012).

Another study attempted to exploit male mosquito behavior through autocidal and autodissemination methods by releasing laboratory-reared male mosquitoes

contaminated with pyriproxyfen (Mains et al. 2015). This approach has been labeled as “Auto-Dissemination Augmented by Males” (ADAM). Field trials have shown that pyriproxyfen-treated males were able to introduce lethal doses to oviposition sites, both in the presence and absence of female mosquitoes (Mains et al. 2015). The big advantage that this approach provides is that male mosquitoes are proficient at finding females and female mosquitoes are adept at finding cryptic larval habitats (Gaugler et al. 2012). In addition, the ADAM method is not dependent on the indigenous populations being targeted, but could rather be deployed in the spring prior to the buildup of native populations.

The sugar feeding behavior of mosquitoes may also be manipulated as a potential control option, i.e., attractive-toxic sugar bait (ATSB) method (Muller et al. 2010, Marshall et al. 2013). The ATSB approach utilizes a sugar source mixed with an insecticide either within a bait station or sprayed on vegetation where mosquitoes may rest and sugar feed (Muller et al. 2010, Xue et al. 2011, Fulcher et al. 2014). Although the primary toxin utilized within the ATSB approach has been boric acid, eugenol, or garlic oil, other insecticides including dinotefuran, pyriproxyfen, and spinosad have also been used (Xue et al. 2011, Marshall et al. 2013, Fulcher et al. 2014).

The effect of spraying a mixture of pyriproxyfen (1 mg/liter) and either 1% boric acid sugar bait or eugenol sugar bait on croton petra plants (*Codiaeum variegatum* L.) was evaluated against the container-inhabiting mosquito, *Ae. albopictus* (Fulcher et al. 2014). Treatments were applied to plants and evaluated against adult and larval *Ae. albopictus* in the laboratory through contact and wash off experiments, respectively. The control treatment was an attractive sugar bait lacking an AI (Fulcher et al. 2014). The

plants treated with ATSB plus the IGR resulted in 60-100% mortality of laboratory-reared adult *Ae. albopictus* (Fulcher et al. 2014). The pyriproxyfen solutions collected from the plant wash experiment resulted in 80-100% emergence inhibition to the exposed third- and fourth-instar larvae, compared with the untreated control (Fulcher et al. 2014). Attractive toxic sugar baits mixed with the IGR provided effective control of adult and larval mosquitoes (Fulcher et al. 2014).

A variety of genetic control methods are currently in development to suppress *Ae. albopictus,* including the sterile insect technique (SIT), insects carrying a dominant lethal gene (RIDL), and *Wolbachia*-induced cytoplasmic incompatibility (CI) (Faraji and Unlu 2016). The SIT exposes males to irradiation or harsh chemicals in the lab that create mutations leading to sterility prior to their release (Faraji and Unlu 2016). However, this method is prone to logistic issues (mass production, separation of males from females prior to release) and often reduced fitness of released males (Alphey 2014). The SIT technique can be further enhanced by incorporating RIDL, which utilizes male mosquitoes to carry and transfer transgenes into wild populations (Faraji and Unlu 2016). A dominant lethal transgene may be inserted and its expression repressed to select the time of death of the offspring, providing much more flexibility with control options and reduced fitness pressures on released males (Alphey 2014). However, regulatory issues and public perception have so far barred the use of RIDL control techniques in the US. Preliminary trials incorporating an SIT technique in Italy showed that weekly release of 896-1,590 sterile males per hectare induced a significant sterility level in the local population of *Ae. albopictus* within the treatment site (Bellini et al. 2013). Five trials were performed in three small towns from 2005 to 2009 where reared

male pupae, were exposed to gamma rays (85 Gy in 2005 to 30-40 Gy in 2008, and to 30 Gy in 2009) and immediately released adults in the field (Bellini et al. 2013). Adult population density was estimated based on a weekly monitoring of egg density in ovitraps, while induced sterility was estimated by measuring the hatching percentage of weekly collected eggs in SIT and control areas (Bellini et al. 2013). When the sterility level achieved values in the range of 70-80%, a similar reduction was found in egg density (Bellini et al. 2013). Monthly mean percent of egg sterility and egg density reduction in SIT (compared to control areas) indicated the absence of any clear effect on reduction of the adult population density when induced sterility was below 50% (Bellini et al. 2013). When sterility levels increased over 50%, adult density was significantly reduced.

Another control measure exploits a group of intracellular organisms known as *Wolbachia,* maternally transmitted bacteria that cause a phenotype known as cytoplasmic incompatibility (CI) in mosquitoes. Sperm from a *Wolbachia*-infected male mosquito are incompatible with eggs from uninfected females or those who are infected with a different *Wolbachia* type, leading to reduced fecundity and fertility (Dobson 2004). Unidirectional crosses may occur between uninfected females and infected males, whereas bidirectional crosses occur between individuals infected with different strains of *Wolbachia* (Dobson 2004). Several strains of *Wolbachia* (wRi, wMelPop, wPip, wMel) have been successfully microinjected into *Ae. albopictus*, paving the way for bidirectional CI control measures within field populations (Xi et al. 2006; Calvitti et al. 2012). The application of *Wolbachia*-infected mosquitoes is only recently being used as a viable mosquito control strategy, but given the vast array of developmental pressures

it exerts on hosts, this method may be enormously beneficial in the battle against container-ovipositing *Aedes*. However, much like other genetic control measures that are still in their infancy and subject to cost and community acceptance, conclusive large-scale field data is needed prior to their establishment within integrated mosquito suppression programs.

Alternative control methods, particularly in the field of genetic control strategies, are attracting interest (Faraji and Unlu 2016). These methods can potentially provide new and species-specific control strategies through the introduction of a heritable trait into the target population for area-wide suppression (Alphey 2014). The best SIT results against mosquitoes have been achieved in isolated island situations, where immigration was not a confounding factor (Patterson et al. 1970). Future field studies involving *Ae. albopictus*, will provide crucial data to evaluate the efficacy of the RIDL technique under different geographic and climatological conditions. Although simulation modeling has suggested that the SIT would be both effective and economical when combating container-inhabiting *Aedes* mosquitoes (Alphey et al. 2011).

How does an insect growth regulator work?

Insect growth regulators may be more effective in controlling target pests/vectors than conventional synthetic pesticides due to their low mammalian toxicity and reduced risks to non-target species (Mian et al. 2017). Growth regulators include chemicals with unique modes of action such as juvenile hormone analogs, chitin synthesis inhibitors, and ecdysone agonists (Soin et al. 2010). Juvenile hormone (JH) agonists mimic the effects of naturally occurring juvenile hormone, if levels of JH remain high, every molt results in insects emerging as juveniles until death occurs (Pfeiffer 2008). Other

potential effects of JH on insects include: sterilization of adults, inhibition of egg hatch and laying of nonviable eggs (Pfeiffer 2008). Some Insect growth regulators containing methoprene do not have good stability in outdoor settings so use is confined primarily to indoor applications such as control of roaches, fleas, and pest affecting stored products; while newer JH agonists such as pyriproxyfen have good stability and are used in exterior applications (Pfeiffer 2008).

Ecdysone agonists mimic ecdysone and force insects to molt prematurely, hence causing death. Other effects of these compounds on insects include increased egg mortality and reduced rates of reproduction (Pfeiffer 2008). The AI from the Neem tree (*Azadiractin indica*) is azadiractin and is extracted primarily from seeds of the tree (Pfeiffer 2008). One effect of azadiractin on insects is to inhibit Prothoracicotropic hormone (PTTH), the hormone which stimulates ecdysone production that initiates the molting cycle (Pfeiffer 2008). Other effects of azadiractin on insects included deformities after molts, reduced growth and antifeeding activity, which is usually short lived (Pfeiffer 2008).

Compared to microbial larvicides and adulticides containing organophosphates and synthetic pyrethroids, IGRs have shown promising results in killing mosquitoes (Ali et al. 1995). Examples of other IGRs that are registered as larvicides are methoprene, pyriproxyfen, and diflubenzuron (WHO 2006). An experiment was conducted on *Ae. aegypti, Ae. albopictus, Ae. atropalpus,* and *Culex pipiens* mosquitoes to test the ovicidal activity of three IGRs: ecdysone agonist (azadirachtin), chitin synthesis inhibitor (diflubenzuron) and juvenile hormone analog (pyriproxyfen) at 0.001, 0.01, 0.1, and 1.0 ppm concentrations (Suman et al. 2013). The same study hypothesized that variations

in egg morphology and oviposition behaviors would determine the ovicidal efficacy of the tested IGRs and that embryonated eggs would be less susceptible to IGRs than freshly laid eggs. Freshly laid eggs were exposed to IGRs by allowing seven gravid females of each species (previously mentioned *Culex* and *Aedes* species) in an oviposition chamber containing previously treated IGR water at the aforementioned concentrations (Suman et al. 2013). The same study showed that egg hatching (*Ae. albopictus* and *Cx. pipiens*) inhibition increased with increased IGR concentration (egg hatching inhibition was calculated as the percentage of unhatched eggs). In *Ae. albopictus,* most eggs hatched at lower concentrations: pyriproxyfen, azadirachtin and diflubenzuron with inhibition rates (% of unhatched eggs) ranging between (1.7-2.8% at 0.001 ppm) and (7.2-10.8% at 0.01 ppm) (Suman et al. 2013). However, freshly laid *Ae. albopictus* eggs exposed to higher concentrations of pyriproxyfen failed to hatch (40.1% inhibition at 0.1 ppm and 80.6% at 1.0 ppm) (Suman et al. 2013). *Aedes albopictus* egg hatch inhibited by pyriproxyfen was significantly higher than eggs exposed to concentrations of azadirachtin (23.6% inhibition at 0.1 ppm and 42.87% at 1.0 ppm). Diflubenzuron treatments at the same concentrations (29.2% inhibition at 0.1 ppm and 35.8% at 1.0 ppm) resulted in lower egg hatching than pyriproxyfen (Suman et al. 2013).

The inhibition of *Cx. pipiens* (Linnaeus, 1758) egg hatching was higher in the group exposed to diflubenzuron (21.8% at 1.0 ppm) than pyriproxyfen and azadirachtin, which were indicated at just <20% in containers with 1.0 ppm (azadirchtin) (Suman et al. 2013). Though higher concentrations (0.1 ppm and 1.0 ppm) of pyriproxyfen, azadirachtin and diflubenzuron inhibited egg hatching, the percent of eggs (*Cx. pipiens)*

that hatched (90%) was higher than the other species (*Ae. aegypti, Ae. albopictus, Ae. atropalpus)* (Coquillett) (Suman et al. 2013).

Pyriproxyfen

Pyriproxyfen is an IGR registered for use in agricultural, aquatic, and commercial settings (EPA, 2011). Found in more than 300 registered pesticide products, pyriproxyfen is commercially applied in and around food storage/handling establishments and other structures (EPA, 2011). Residential uses include applications inside the home such as on pets and in their living areas to control fleas and ticks, outside on gardens, lawns, patios, and other structures to control mosquitoes and other insects (EPA, 2011). Aquatic uses may include ornamental ponds to eliminate mosquito larvae, waste water or settling ponds, sewers, and other water-harboring sites that do not drain into natural bodies of water (EPA, 2011). Pyriproxyfen is not thought to be toxic to adult insects, but it inhibits egg-laying and egg-hatching and prevents young insects (fleas, cockroaches, ticks, ants, carpet beetles, and mosquitoes) from growing into adults (Hallman et al. 2015).

Pyriproxyfen is nontoxic to birds, mammals, and adult honeybees; however, honey bee eggs and larvae may experience delayed growth (Hallman 2015). Pyriproxyfen is not identified as a cause of impairment for any water bodies listed as impaired in section 303(d) of the Clean Water Act (EPA, 2011). However, the specimen label for the formulated product Archer (AI: pyriproxyfen) states that the product is toxic to fish and aquatic invertebrates, therefore, it cannot be applied directly to bodies of water or where surface water is present. Pyriproxyfen can be moderately to highly toxic in some species of fish; however, two species of fish exposed to pyriproxyfen-treated

water showed no toxic effects, even at the highest labeled dose (Hallman et al. 2015). Pyriproxyfen has a moderate potential to bioaccumulate in fish because it can be stored in fat but it is difficult to tell how toxic this chemical is to fish because it dissolves poorly in water (Hallman et al. 2015). Since pyriproxyfen binds tightly to soil particles and does not dissolve easily in water, it is not likely to navigate through the soil and contaminate ground water; although it may contaminate surface water through spray drift, erosion, or agricultural runoff (Hallman et al. 2015). Many products containing pyriproxyfen are used in agriculture; however, when applied to plants, there is evidence pyriproxyfen can move within leaves but it does not move throughout plants easily (Hallman et al. 2015).

Lambda-cyhalothrin

Many pyrethroid pesticides (e.g., cypermethrin, permethrin, fenvalerate, tetramethrin) are potential endocrine disrupting chemicals and may have a negative effect on the reproductive and immune systems of animals and humans (Bian et al. 2004; Pine et al. 2008). One of the most common pyrethroids is lambda-cyhalothrin, which is a highly effective insecticide even at low doses and widely used in home, agriculture, and hospitals worldwide (Tukhtaev et al. 2012). Examples of product formulations for lambda-cyhalothrin include wettable powders, pellets, emulsifiable concentrates, solutions, and slow release microencapsulate suspensions (WHO 2015). Lambda-cyhalothrin has a low toxicity in birds; however, it is highly toxic to fish and has the potential to accumulate within aquatic invertebrates (WHO 1990). Lambda-cyhalothrin is highly toxic to bees when ingested or if external contact with the chemical occurs (WHO 1990). A representative soil half-life for lambda-cyhalothrin is 30 days with values ranging from 28-84 days (Hornsby et al. 1995). In a field study, lambda-

cyhalothrin degraded with a half-life of approximately nine days (Hill and Inaba, 1991). The low water solubility and high binding affinity of lambda-cyhalothrin indicates a low potential to contaminate ground water (Vouge et al. 1994)

Demand CS uses an encapsulated process that slowly degrades over time to shield the AI (lambda-cyhalothrin) from UV rays, pH extremes and absorption into porous surfaces, so it remains intact longer (Syngenta 2014). iCAP technology packs up to 10,000 capsules of insecticide into every treated square inch (Syngenta 2014). According to the Syngenta product label, Demand CS effectively controls over 30 different types of insects, including: ants, bedbugs, millipedes, bees, mosquitoes, beetles, centipedes, cockroaches, scorpions, silverfish, crickets, spiders, fleas, ticks, flies, and wasps (Syngenta, 2014).

Lambda-cyhalothrin is a Type II pyrethroid that is used in barrier sprays for adult mosquito control. Leaves treated with lambda-cyhalothrin were used in a laboratory bioassay against Ae. aegypti (Muzari et al. 2014). This study demonstrated high (> 94%) knockdown after 1 h of exposure to lambda-cyhalothrin and 100% mortality after mosquitoes were held for 24 h in a clean container. Lambda-cyhalothrin (Demand®, 25 g active ingredient [AI]/L) applied as a barrier spray in Australia showed a significant decrease in mosquito populations (primarily Verallina lineata Taylor) (measured using sweep net collections) between treated and control sites (Muzari et al. 2014).

Barrier Sprays

To test the efficacy of lambda-cyhalothrin on *Ae. albopictus* mosquitoes, a study in China utilized the barrier spray formulated product Demand CS was applied to vegetation at the recommended concentration of 20 ml/liter, while nothing was applied

in the control site (Li et al. 2010). Applications were made to vegetation using a backpack power sprayer (model MD6026, Maruyama Mfg. Co. Inc., Tokyo, Japan) operated at an output rate of 40 ml (20 mg AI)/m^2. Treatment was applied to lower surfaces of vegetation around the perimeter of the residential yards (Li et al. 2010). Human landing counts were used to assess differences in abundance of mosquitoes between treatment and control properties. Overall, 83–98% reduction in *Ae. albopictus* was achieved in the area treated with lambda-cyhalothrin treated during nine weeks of post-treatment observations (Li et al. 2010). The lambda-cyhalothrin barrier spray on vegetation resulted in 96% reduction of the adult mosquito population in treated yards on the first day after treatment. Within the first week posttreatment, *Ae. albopictus* landing rates were reduced by 98%, compared with the untreated control site (Li et al. 2010). At four and nine weeks posttreatment, the reduction in *Ae. albopictus* populations was 88% and 95%, respectively (Li et al. 2010). Based on these findings, lambda-cyhalothrin applied as a barrier treatment to vegetation and lower plant canopy can be effective in reducing mosquito landing rates for up to two months (Li et al. 2010).

Insecticide resistance

Insecticide resistance can be associated with mutations in the sequence of the target protein that induce insensitivity to the insecticide (target-site resistance), and/or to the up-regulation of detoxification enzymes (metabolic-based resistance) (Marcombe et al. 2014). Exposure to insecticide can result in physiological resource allocation to reproduction at the expense of survival, which indirectly accelerates pest population growth and can lead to resurgence and secondary pest outbreaks (Bong et al. 2017). Energy allocation in insecticide-resistant populations may follow a different pattern, as

resistant insects have been shown to exhibit a series of biological trade-offs in reproduction, somatic maintenance (processes to stay alive), or behavior (Martins et al. 2012; Brito et al. 2013). This phenomenon is a consequence of a metabolic exchange in which energy and resources that usually go toward reproduction and fitness are channeled to insecticide detoxification (Rivero et al. 2011). In general, insecticide-resistant insects exhibit low biological performance compared to susceptible individuals (Bong et al. 2017). If resistant insects increase their reproductive effort when mortality risk increases, an increase in the resistant population in the field could potentially occur (Bong et al. 2017).

Due to the extensive use of adulticides, some mosquito populations have developed resistance against many classes of insecticides (Brogdon and McAllister 2004). Unlike *Ae. aegypti*, which has developed resistance to multiple insecticides worldwide, *Ae. albopictus* has shown a comparably low level of resistance to insecticides (Hemingway et al. 2004). Conversely, in Thailand, there appeared to be increased resistance of *Ae. albopictus* to permethrin, malathion and temephos has been reported (Ponlawat et al. 2005). Vector control of *Ae. albopictus* is difficult, as observed in Italy, the USA and even in France, where the species continues to spread (Scholte and Schaffner 2007). These failures generally can be attributed to a lack of knowledge of the insect vector ecology and non-surveillance based vector control strategies (Fontenille et al. 2007).

Larvicides are effective when applied directly to oviposition habitats, but treatment of some container habitats is impractical because they are too numerous and often obscure in the environment (Faraji and Unlu 2016). It is important to continue

monitoring mosquito populations for insecticide resistance to ensure that adulticides being used remain efficacious (Faraji and Unlu 2016). Insecticides remain the primary tools used against container-ovipositing *Aedes*, hence there are ongoing concerns about insecticide safety, cost, public perception, efficacy, and other potential environmental impacts (Faraji and Unlu 2016).

Container water level effects on oviposition

The successful invasion of *Ae. albopictus* is tied to its ability to take advantage of artificial container habitats in peridomestic environments (Hawley 1988). Urban environments usually contain artificial and natural mosquito habitats; however, the volume of water in containers and size of containers varies across fine spatial scales (Leisnham and Slaney 2009, Yee et al. 2012). Precipitation is necessary to fill container habitats and maintain water resources necessary for larval development (Alto and Juliano 2001, Unlu et al. 2014). Homeowners can also provide water for containers that promote mosquito growth (e.g., watering plants with receptacles underneath). In times of low precipitation, smaller containers are quick to dry out and may be too transient to allow larval development (Barlett-Healy et al. 2012, Becker et al. 2012). However, larger permanent containers (tanks, drums, jars) that are either more closely linked to human water storage or retain water for longer are more suitable larval habitats for *Ae. albopictus* (Unlu et al. 2011, Becker et al. 2014).

In the northeastern US, *Ae. albopictus* are found more often in medium volumes of water (250 mL - 1L) inside of buckets, pans, and tires and rarely in small volumes of water (40 -250 mL) found in trash items such as discarded cups and cans (Unlu et al. 2013). Discarded tires are an important *Ae. albopictus* larval habitat and hold an

average of 1 L of water (Schreiber et al. 1992). This species is less prevalent in large (>1 - 20L) and very large (> 20L) volumes of water such as those found in abandoned swimming pools and backyard ponds (Barlett-Healy et al. 2012, Unlu et al. 2013). The variety and abundance of larval habitats, coupled with cryptic and hard to reach habitats such as corrugated extension spouts, requires a level of control that is not currently possible within most, if not all, mosquito control programs (Faraji & Unlu, 2016). Therefore, it is crucial to encourage more surveillance and research efforts for control of *Ae. albopictus* (Paupy et al. 2009).

The current study utilizes field and laboratory methods to evaluate the impacts on *Ae. albopictus* life table characteristics when exposed to the IGR pyriproxyfen. In the field, we evaluate the extent to which different application rates and frequencies of a barrier spray containing Demand CS® (AI: lambda-cyhalothrin) with Archer® (AI: pyriproxyfen) impacts life table characteristics (fecundity, fertility, adult emergence rates) of *Ae. albopictus* in a suburban environment. A separate laboratory study was conducted using Archer® as a simulated barrier spray to examine the impact of pyriproxyfen on *Ae. albopictus* fecundity, fertility, and eclosion.

CHAPTER III – MATERIALS AND METHODS

This section describes two separate studies evaluating the impacts of insecticides on life table characteristics of *Ae. albopictus*: 1) Field barrier spray exposure of mosquitoes to AIs lambda-cyhalothrin and pyriproxyfen, and 2) Simulated barrier spray exposure of mosquitoes to pyriproxyfen under controlled laboratory conditions.

Results from the field evaluation of control measures are under review with a peer-reviewed journal. Here, we primarily discuss the results of the life table characteristics assessment of Ae. albopictus for the field and laboratory study.

Field Barrier Spray Exposure

Recruitment of Participants

Mosquitoes were collected from a suburban neighborhood (Cherry Oaks) in Pitt County, North Carolina on a weekly basis from May 16 – November 2, 2017. Properties were recruited for the study based on the presence of foliage appropriate for barrier sprays. Control properties were required to be at least one property away from a treatment property (i.e., not sharing a border with a treatment property). Investigators initially went door-to-door to recruit participants in person. Homeowners who were present at the time of the visit were provided verbal and written information on the study. For residents not present, a handout was left at their front door with information about the study and contact information for the principal investigator. Two to three follow up visits were conducted until homeowners were contacted and informed of the study. A consent form was signed by each participant that granted permission for investigators to enter the yard once a week to set and retrieve mosquito traps. Barrier spray services

were provided to participating residents free of charge for the duration of the study. Residences (N=12) were grouped in clusters of three and separated into three treatment groups and a control group. Homeowners were blinded to which treatment was applied to their property and notified in advance the dates that treatments would take place.

Study Area

Certified pest control operators from Clegg's Pest Control (private company with franchise location in Greenville, NC; http://www.cleggs.com/) carried out barrier sprays for the project and treated foliage on properties using a Stihl SR 200 backpack blower mister as described below. Foliage, vegetation, and shrubs on treatment properties were sprayed every 30 to 60 days depending on treatment group. No treatments were conducted on control properties. Plant life was similar among treatment and control residencies however, this was not quantified.

The label recommends Demand® CS be applied at the 0.06% rate for residual control of mosquitoes and here, we used this rate at an interval of 60 d and a lower rate (0.03%) at a more frequent interval (30 d) in order to evaluate efficacy. Similarly, the label recommends Archer® be applied at the 0.010% rate for residual control of mosquitoes and here, we used this rate at an interval of 60 d and a lower rate (0.005%) at a more frequent interval (30 d) in order to evaluate efficacy. Label instructions were followed and operators applied 2-5 gallons of the finished solution per 305 m2 in circular patterns to vegetation until runoff. Treatments were not conducted in high winds or misting/rainy conditions. We coordinated with the Pitt County Vector Control Manager and the City of Greenville Public Works mosquito control operators to alert them of the

ongoing study and requested that no insecticides be sprayed in the study area for the duration of the project.

Description of Treatments

Four groups of three properties/group (N=12 properties total) received the following treatments from May 16 – November 2, 2017:

1) Demand CS 0.03% + Archer 0.005% every 30 days (treatment dates: June 13, July 13, August 15, September 15, October 17),
2) Demand CS 0.06% + Archer 0.010% every 60 days (treatment dates: June 13, August 15, October 17),
3) Demand CS 0.03% every 30 days (treatment dates: June 13, July 13, August 15, September 15, October 17),
4) Control (no treatment).

Pitt County Vector Control and the City of Greenville Public Works mosquito control operators were notified of the ongoing study and refrained from spraying insecticides in the neighborhood during the period of the study.

Oviposition Intensity

A black plastic ovitrap (500-ml) was half-filled with tap water and securely zip-tied to a shepherd's hook at each household (N=12) to monitor oviposition intensity among treatment and control sites. Seed germination paper (2.5 x 7 cm) was utilized as oviposition substrate and clipped inside of the half-filled cup, with holes for drainage drilled 4 cm from the top of the cup. Oviposition substrates from each property were collected and replaced weekly throughout the duration of the study. Each week, egg strips were transported to the laboratory to obtain egg counts (fecundity) and identify

species of eggs present using a dissecting microscope. Each week, water remaining in cups was poured into Whirl-Pak® bags (labeled with property address) and replaced with fresh tap water. Collection bags were transported to the lab and any existing larvae were reared to adult, identified to species, and counted. Occasionally, eggs hatched in the cups before weekly egg strip collections, so this process ensured that all hatched/unhatched eggs and larvae could be accounted for in the data. Data was coded for each property and entered into a spreadsheet for future analysis.

Assessment of Life Table Characteristics

After eggs were counted on egg strips each week, egg strips were allowed to completely dry overnight (to stimulate hatching) before being transferred to emergence cages (BioQuip, Rancho Dominguez, CA). Egg strips were submerged in 450-750 mL of tap water in emergence cages, fed liver powder *ad libitum,* and incubated at 28°C with a 14h:10h (light:dark) cycle. Approximately five days post submergence, larvae were quantified for each property to measure fertility (fertility= number of larvae hatched/number of eggs on ovistrips). Adults that emerged were killed by freezing, separated into petri dishes by ovistrip, identified to species, counted, and recorded.

Laboratory Simulated Barrier Spray Exposure

Mosquito Rearing

The eggs of an existing *Ae. albopictus* (generation F_{30} originally from Louisiana) colony were submerged in sixteen pans (24 cm × 36 cm × 5 cm) (to reach target goal of 1000 females) each containing 700 mL of dechlorinated water and fed liver powder. Pans were housed in an incubator at 28°C with 14h:10h (light:dark) cycle. Larvae were fed liver powder *ad libitum* for the duration of their growth cycle. Pupae were transferred

to a (150 mL) plastic cup half-filled and placed into metal mosquito cages (30 cm^3) prior to adult emergence. Batches of pupae were transferred in this manner for approximately five days until all larvae had developed into pupae. Adults emerging in the cage were provided with a 20% sugar solution *ad libitum*.

Mosquito Blood Feeding

Four to five day old adult female *Ae. albopictus* mosquitoes were transferred to eight 1 L cardboard cages (125 mosquitoes/cage) with mesh screen and provided with a 20% sugar solution *ad libitum*. The sugar solution was removed and replaced with water 24 h before blood feeding to improve feeding rate. Mosquitoes were blood fed with a Hemotek Membrane Feeding System (Hemotek Limited, England) using a BG human scent lure to stimulate feeding. After a 1 h feeding period, mosquitoes were immobilized with cold and fully engorged females were transferred to two separate 1 L cardboard cages (50 mosquitoes/cage). Mosquitoes were transferred to a 28°C incubator and provided a 20% sugar solution *ad libitum* until further processing.

Pyriproxyfen Exposure of Blood Fed Mosquitoes

Control (Acetone only) and treatment (Archer® solution made in acetone: AI pyriproxyfen) solutions were prepared. Field application label recommendations were used to determine the dose (7.49 g/L) of Archer® liquid stock we used. Glass Wheaton bottles (250-ml) and lids were completely coated with either 1 mL acetone (control) or 1 mL of Archer stock (treatment). Glass bottles with their caps removed were placed on a bottle roller until the contents evaporated (1-2 minutes), leaving a film of pyriproxyfen in the treatment bottles and clean control bottles. Once the bottle coating procedure was

completed, uncapped bottles were placed into a dark drawer to prevent light degradation and were used within 24 hours.

Twenty-four hours post-blood feeding, blood fed mosquitoes (50 for treatment, 50 for control) were transferred into prepared glass bottles for a 2 h exposure period. Twenty-five mosquitoes were placed in each respective bottle. Bottles were rolled 180° every 30 minutes (total of three times) to ensure mosquito tarsi were exposed as they would be on foliage in the environment when resting after a blood meal and/or while sugar feeding.

For both Archer® and control groups, either small (59 mL) or large (177 mL) black plastic oviposition cups were hot glued into 1 L cardboard cages with mesh as follows: 1) small ovicup, Archer®, 2) large ovicup, Archer®, 3) small ovicup, control, 4) large ovicup, control. A total of 88 1 L cardboard cages (N=22 per group) were created. Clear plastic ovicups (59 mL or 177 mL) were placed inside the black ovicups for ease of removal and counting larvae later. Ovistrips (small: 13 x 4.3 cm, large: 18.8 x 5.4 cm) were placed in ovicups to ensure that roughly half of each strip was submerged in the water and the other half was above the water. Each cage was coded by treatment group and oviposition cup size. A single blood fed mosquito was transferred to each cage and provided a 20% sucrose solution *ad libitum*. Liver powder was provided to larvae *ad libitum*.

Six days post-blood feeding, each adult mosquito was removed from its cage (noted as dead or alive) and its ovaries were dissected using a dissecting microscope to enumerate eggs that may have been retained. Egg strips were retrieved and eggs were counted to obtain the fecundity rate. Egg strips were dried and then placed back into the

same coded (by specific cage number) oviposition cup and returned to its respective 1L cardboard cage. At six and twelve days after egg strips were submerged, larvae were counted in oviposition cups to track fertility rates (number of larvae hatched/number of eggs laid)*100. All adults (females and males) that emerged were killed by freezing, counted, and recorded for the duration of the study to examine the emergence rates (number of adults emerged/number of larvae hatched)*100.

Statistical Analyses

For the field study, data analyses were conducted using SAS (SAS Institute, Cary, NC) and comparisons with $P < 0.05$ considered significant. A mixed model (PROC MIXED) using repeated measures (traps) (control properties used as a reference) was used to determine the extent to which *Ae. albopictus* eggs (fecundity), larvae (all species) (fertility), adults that had emerged in the laboratory (all species and *Ae. albopictus*) varied between treatments and weeks. Analyses of treatment effects were conducted after treatments had commenced (> epidemiological week 24; mid-June).

For the lab study of our simulated barrier spray using pyriproxyfen, data analyses were conducted using SPSS. Descriptive statistics were used to compute means, standard error, and standard deviation of each variable (fecundity, fertility, and adult emergence). Analysis of variance (ANOVA) and a test of homogeneity of variances was conducted to determine the extent to which survival rate, fecundity, and fertility of exposed females, as well as adult emergence rates of progeny differed between treatments and ovicup sizes. A Bonferroni correction was applied to account for multiple comparisons.

CHAPTER IV – RESULTS

In these separate (lab and field) studies, we evaluated the impacts of insecticides on life table characteristics of *Ae. albopictus*: 1) Field barrier spray exposure of mosquitoes to Demand CS (AI: lambda-cyhalothrin) with Archer (AI: pyriproxyfen), and 2) Simulated barrier spray exposure of mosquitoes to pyriproxyfen under controlled laboratory conditions.

Field Barrier Spray Exposure Experiment

Results from the field evaluation of control measures are under review with a peer-reviewed journal. Here, we primarily discuss the results of the life table characteristics assessment of Ae. albopictus for the field and laboratory study.

Ae. albopictus **eggs**

From May 16, 2017 – November 2, 2017 a total of 4,423 *Ae. albopictus* eggs were collected from ovitraps. Means of *Ae. albopictus* egg abundance per trap (for each treatment) are shown in Figure 2. Significant differences were observed in the abundance of *Ae. albopictus* eggs between treatments (df = 3; $F = 4.62$; $P = 0.037$) (Figure 2) with control lots having higher mean numbers of eggs than treatment lots. Conversely, no significant differences were observed in egg abundance between weeks (df = 19; $F = 1.05$; $P = 0.412$) (Figure 3).

Data for eggs collected from the field and reared to adult in the laboratory are shown in Figures 3 and 4. Significant differences were observed in the mean numbers of larvae hatched per ovitrap (fertility) between treatment groups (df = 3; $F = 4.32$; $P = 0.043$). Significantly more larvae (all species) hatched from eggs on strips collected from Control lots, compared to other groups. A similar pattern was observed in the

mean numbers of *Ae. albopictus* adults (females and males that were reared in the laboratory from egg strips collected in the field) (df = 3; $F = 2.82$; $P = 0.041$) and total adults (all species) (df = 3; $F = 4.04$; $P = 0.050$) between treatment groups wherein significantly more adult *Ae. albopictus* and adults (all species) emerged in the Control group, compared to other groups.

The number of *Ae. albopictus* eggs collected could be predicted by average rainfall four weeks before collections in Control ($P = 0.013$) and DA30 ($P = 0.014$) lots and by temperatures three weeks before collections in DA60 ($P = 0.026$) lots. No other significant relationships were observed between weather variables and *Ae. albopictus* abundance.

Laboratory Simulated Barrier Spray Exposure Experiment

In the pyriproxyfen-exposed treatment group, ≤ 25% of blood fed mosquitoes laid at least one egg, while ≤ 48% of blood fed mosquitoes in the control group laid eggs. Significantly lower (P=0.0008) fecundity was observed in treatment mosquitoes exposed to pyriproxyfen (mean±SE), i.e., (small container: 25.2 ± 7.1 eggs; large container: 24.3 ± 7.1 eggs, compared to control mosquitoes (small container: 49.2 ± 7.8 eggs; large container: 52.7 ± 5.2 eggs) (Figure 6). Regardless of treatment, no significant differences in fecundity were observed between mosquitoes allowed to oviposit in different sized containers.

Hatch rate was significantly lower in the treatment group (compared to control) and was impacted by size of container (P=0.032) and treatment (df=3, F=14.73, P< 0.0001) (large, control: 61.9% ± 7.8; small, control: 38.0% ± 7.1; small, treated: 2.9% ± 1.9; large, treated: 10.3% ± 2.4) (Figure 7). Adult emergence rates were significantly

lower in the treatment group (pyriproxyfen) compared to control group (acetone) and was also impacted by the size of container and treatment (df=3, F=15.58, P<0.0001) (Figure 8).

CHAPTER V – DISCUSSION

Field Barrier Spray Exposure

Significantly more *Ae. albopictus* eggs were observed in Control lots, which shows that treatments may have negatively impacted egg-laying *Ae. albopictus*; however, as expected, these effects varied across weeks. The greater number of hatched larvae per ovitrap and *Ae. albopictus* adults emerging from ovistrips collected in Control lots is logical since these lots were not treated. We expected the lowest number of larvae and emerged adults in the lots receiving high frequency treatments every 30 days with the pyrethroid and IGR (DA30) and this group was equivalent to larvae derived from lots with lower insecticide application frequency (but higher concentration) (DA60) of the IGR. It could be feasible to treat with a higher concentration of Demand® CS with Archer® less frequently, depending on labor and other constraints of mosquito control applicators.

Mosquito abundance can vary based on weather variables and other unknown factors. In field studies, there is also likely year to year variation in mosquito populations that would need to be considered and analyzed. There may even be differences in levels of insecticide susceptibility/resistance in mosquito populations within the same season and that was not addressed in the current study. Mosquito abundance is expected to vary over time under different biological and environmental conditions. Rainfall four weeks prior (Control and DA30 properties) and temperatures three weeks prior (DA60) to trapping was predictive of *Ae. albopictus* eggs. The fact that the lowest number of hatched larvae and *Ae. albopictus* adults that emerged came from the D30 group [treated every 30 days with Demand® CS (no IGR)] is interesting. This may

illustrate some degree of natural variation in *Ae. albopictus* abundance between lots and/or that the adulticides impacted egg laying and/or hatch rates. The reason for assessing life table characteristics (i.e., fecundity, fertility) for eggs laid in the field in the different control and treatment properties was to determine if the IGR and/or adulticide impacted egg laying or hatching. While it would be difficult to ascertain the degree to which mosquitoes from adjacent untreated properties laid eggs in our ovitraps, we see this as a starting point to evaluating this IGR/adulticide mixture used in barrier spray applications.

Laboratory Simulated Barrier Spray Exposure

A laboratory simulated barrier spray exposure was conducted to further analyze the relationship between pyriproxyfen exposure as adults and subsequent measures of fecundity and fertility. It is interesting to note that, after the initial exposure to pyriproxyfen, more mosquitoes died and/or produced no eggs in ovaries or laid eggs among the treatment compared to the control groups. The control group experienced little to no adult mortality (percentage) post-exposure. This raises the question whether pyriproxyfen can be used to control the adult mosquito population.

Fecundity was significantly lower among treatment (exposed to pyriproxyfen) compared to control mosquitoes, i.e., mosquitoes in the control group had almost double the eggs compared to the treatment group. Hence, pyriproxyfen reduced fecundity. Fertility rate was significantly lower in the treatment group and was significantly impacted by the size of oviposition container (amount of water) and treatment. However, adult emergence rates were higher among the control group compared to the treatment group and emergence was significantly higher among large

containers. This raises the question whether or not the concentration of pyriproxyfen in the water was affected by container size and water volume, i.e. larger volumes of water would potentially dilute the effect of pyriproxyfen on larval development. This should be quantified in future studies to understand how much pyriproxyfen is picked up during adult mosquitoes' initial exposure to treatments.

Exposure to the IGR pyriproxyfen reduced fecundity, fertility, and subsequent adult emergence. However, not all mosquitoes exposed to pyriproxyfen experienced the same degree of reduction in life table characteristics measured here. Comparisons should also be done to evaluate efficacy of pyriproxyfen on life table characteristics of other mosquito species (such as *Cx. pipiens/quinquefasciatus*, *Ae. aegypti* (Linnaeus,1763) *Ae. triseriatus* (Say, Thomas. 1823)). We expect variation in these relationships between species, populations, and under different environmental conditions.

CHAPTER VI – CONCLUSION

These findings strengthen the assumption that temperature, rainfall, and abundance of containers in the landscape and other unknown factors, in part, drive *Ae. albopictus* abundance and could influence the efficacy of barrier treatments due to degradation of AIs with environmental pressure and ubiquitous oviposition sources. There is likely variation in abundance of water-holding containers, influence of neighboring properties, and other unknown factors that were not assessed here.

Pyriproxyfen may be a useful control method for some populations of *Ae. albopictus*, especially where resistance to other AIs or cryptic oviposition sources are present. Comparisons should be done to evaluate the efficacy of autodissemination stations, barrier sprays, and/or other methods of application for this AI. In addition, the size, level of organic content, occurrence/abundance of water-holding containers in the landscape could be assessed over the mosquito season to test the efficacy of pyriproxyfen at controlling mosquitoes in a variety of container types. In conclusion, the data gained from these studies can be used to inform mosquito control operators about the efficacy of barrier sprays against *Ae. albopictus.*

To enhance *Ae. albopictus* control, mosquito control personnel should practice source reduction or remove/empty water-holding containers during each visit to the property and inform homeowners of how to eliminate mosquito oviposition sites. Individual homeowners and/or homeowner's associations may consider implementing neighborhood education campaigns to inform all homeowners about preventable mosquito issues. These education and source reduction practices, along with barrier

treatments can be used together as part of an integrated mosquito management approach to prevent/reduce nuisance mosquitoes and protect public health.

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Figure 1. Aerial view of study area. Dotted outlines represent lots included in the study for A) Demand CS 0.03% + Archer 0.005% (every 30 days) B) Demand CS 0.06% + Archer 0.010% (every 60 days) C) Demand CS 0.03% (every 30 days) and D) Control. White circles indicate BG Sentinel and oviposition traps and numbers indicate house address.

Figure 2. Mean numbers (± standard error) of *Ae. albopictus* eggs per trap in different treatment areas. Means with different letters indicate significant differences ($P < 0.05$).

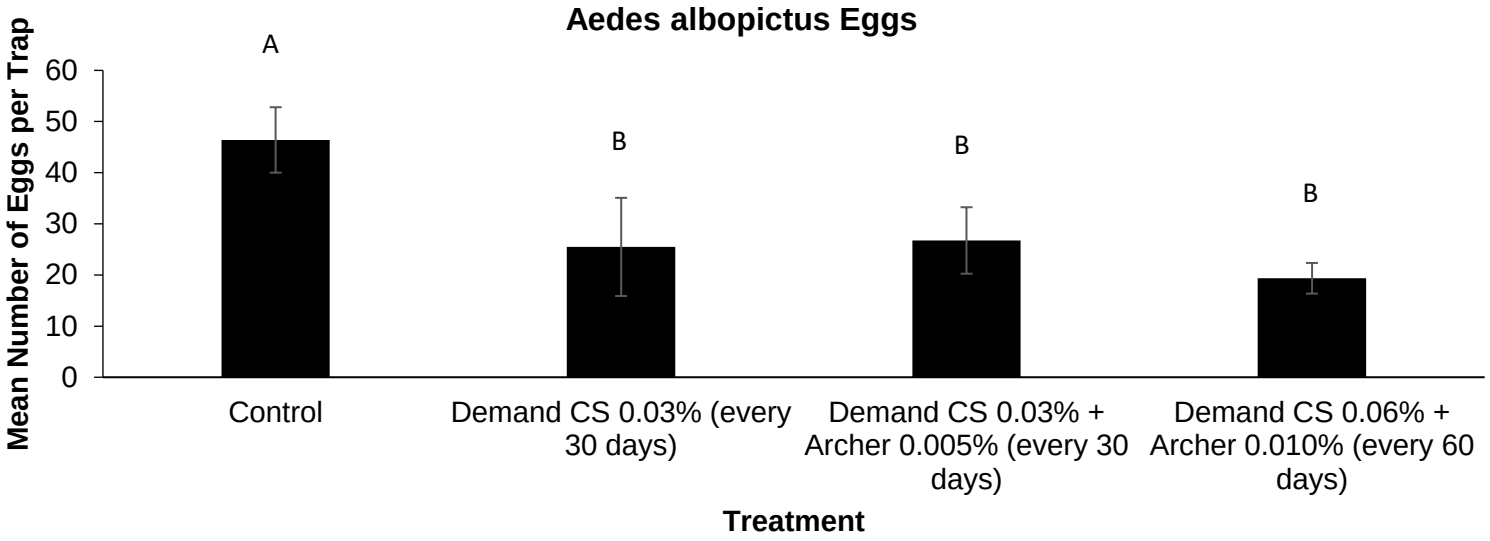

Figure 3. Weekly means (± standard error) of *Ae. albopictus* eggs collected in ovitraps. Red arrows indicate treatment dates.

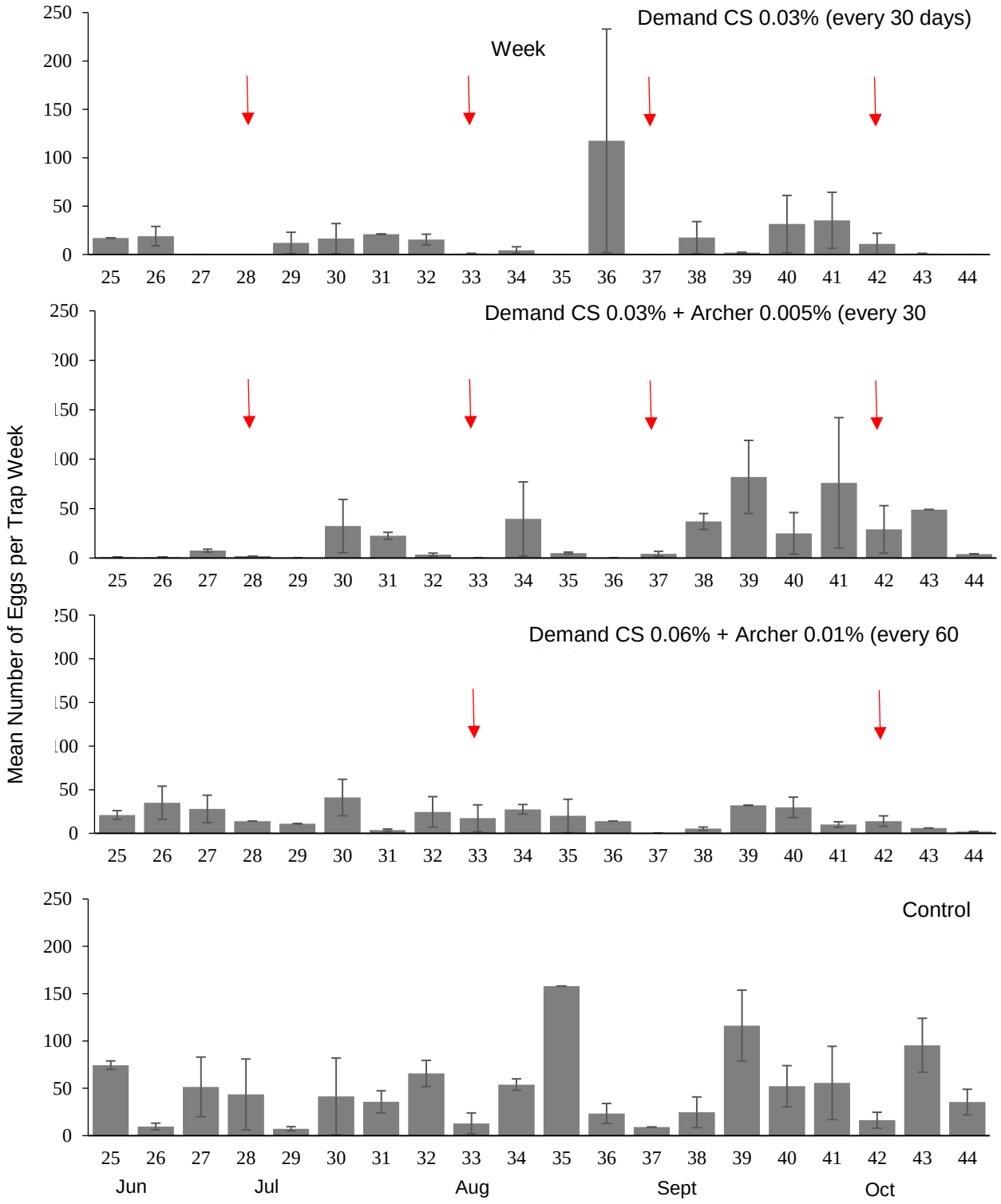

Figure 4. Mean numbers of *Ae. albopictus* eggs on ovistrips, larvae hatched (all species), *Ae. albopictus* adults emerged, and total adults emerged (all species) collected in ovitraps and reared in the laboratory. Means with different letters indicate significant differences within variables ($P < 0.05$).

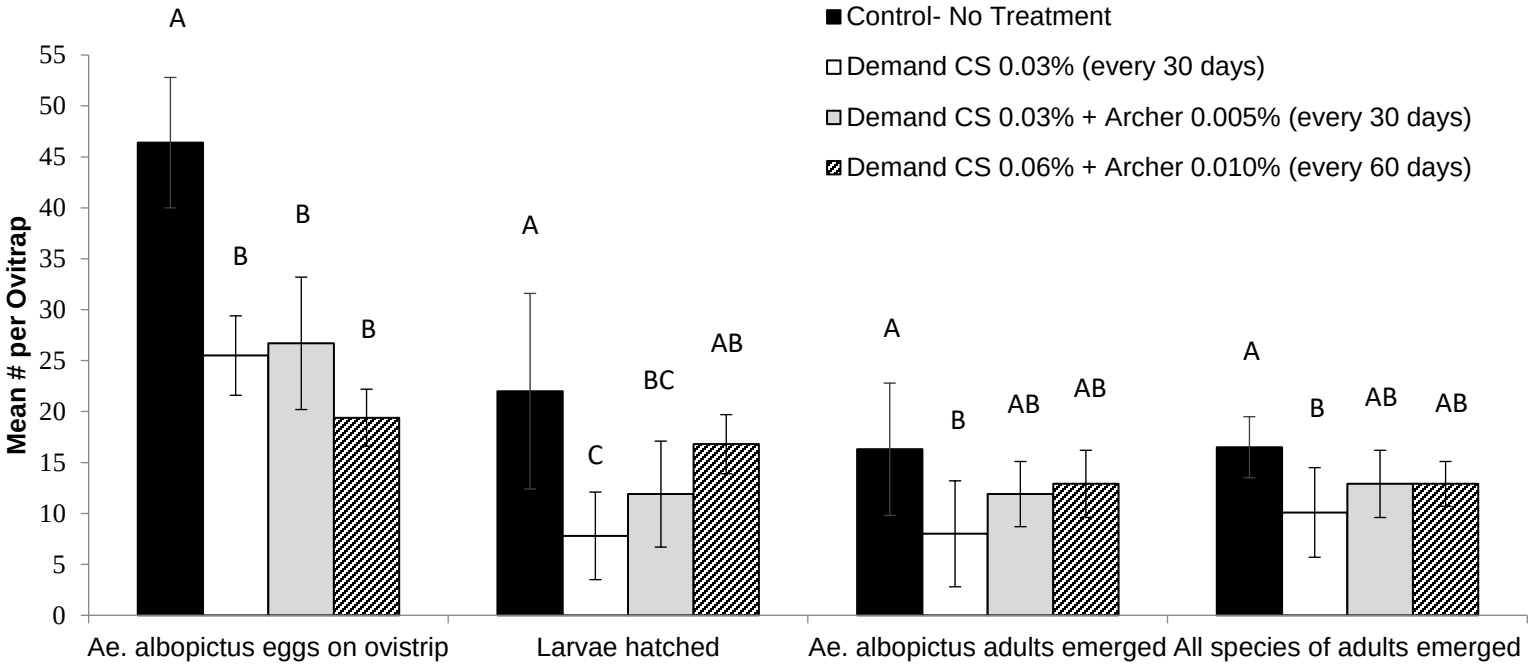

Figure 5. Survival of *Aedes albopictus* adults classified as laying eggs or not laying eggs for those who laid eggs and those who did not lay eggs by treatment group (pre-dissection). Survival was quantified six days post exposure to treatment (archer or control).

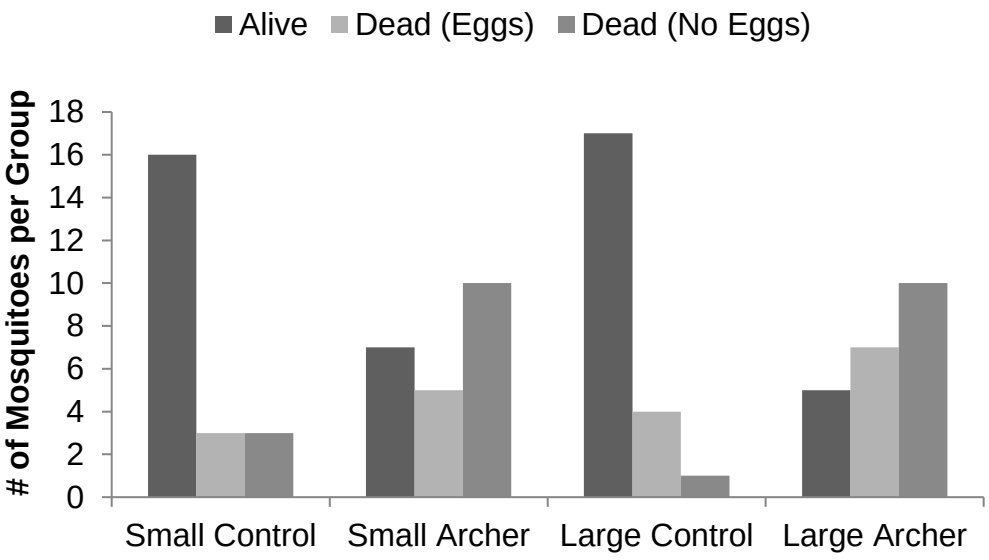

Figure 6. Mean numbers (± standard error) of eggs (fecundity) in *Ae. albopictus* mosquitoes exposed to Archer (AI: pyriproxyfen) compared to control group (acetone).

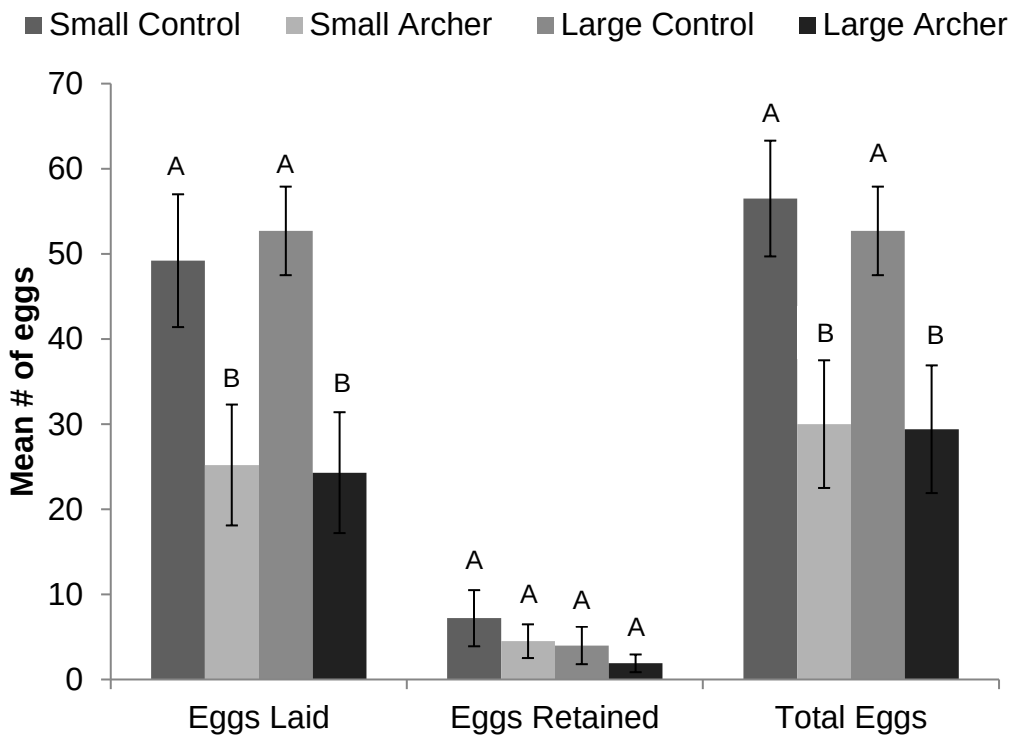

Figure 7. Hatch rate (% ± standard error) of *Ae. albopictus* treatment group (Archer; AI: pyriproxyfen) compared to control group (acetone) in small (57mL) and large (117mL) containers.

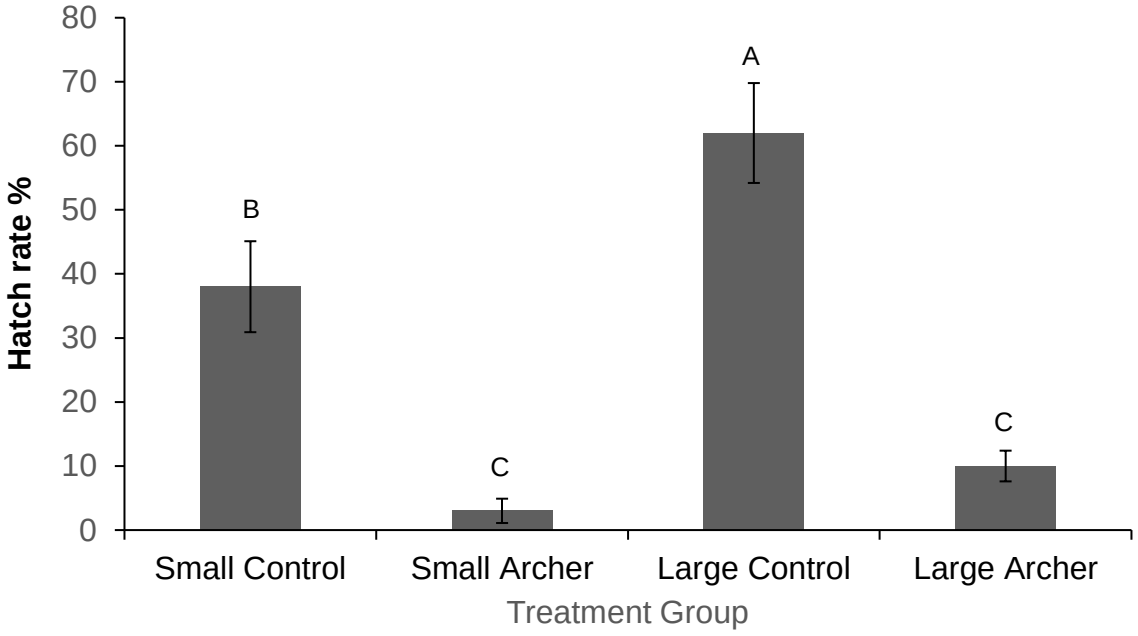

Figure 8. Adult emergence in offspring of *Ae. albopictus* exposed to treatment (Archer; AI: pyriproxyfen) compared to control (acetone) in small (57mL) and large (117mL) containers.