Factors Influencing Pyrethroid Barrier Spray Effectiveness Against *Aedes* Mosquitoes

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Academic Abstract

The Asian tiger mosquito, *Aedes albopictus* (Skuse), is a worldwide nuisance pest that is capable of vectoring several viruses of public health concern. This invasive mosquito has recently expanded its habitable range through its utilization of artificial breeding sites, often due to the activity of humans. These factors, combined with additional expansion due to global changes in climate, have led to invigorated efforts to mitigate the impact of *Ae. albopictus.* Because it is a diurnal species, standard mosquito control efforts utilizing spray trucks or planes to administer insecticides offer little control, as these methods are directed towards crepuscular species. Barrier spray applications, however, have been shown to achieve a significant reduction in local mosquito pressure while requiring less insecticide application. The design behind barrier sprays is to apply insecticide treatments only around areas of interest, instead of trying to eradicate the local population of mosquitoes.

These studies were conducted to evaluate the efficacy of different pyrethroid barrier treatments against *Ae. albopictus* mosquitoes, and to examine the impact of the most effective treatment on local mosquito populations when applied to suburban residences. Three pyrethroids were examined in these studies: Demand® CS (lambda-cyhalothrin), Talstar® Professional (bifenthrin), and Suspend® Polyzone® (deltamethrin). The following factors affecting pyrethroid barrier treatments showed significant impacts on the knockdown and mortality rates of *Ae. albopictus* mosquitoes: the plant species, the label rate at which treatments were applied, the active ingredient used in the treatment applications, the time of exposure to the treated foliage, the presence/absence of a blood meal in the mosquito, and the time after treatment. Demand CS treatments showed the highest proportions of knockdown and mortality in adult female *Ae. albopictus* mosquitoes and did so for the longest amount of time, regardless of the length of the exposure time. Because the Demand CS formulation of lambda-cyhalothrin was shown to be the most effective treatment in the previous studies, it was applied as a barrier treatment to suburban residences in Roanoke, Virginia, in a field trial. Applications of Demand CS as a barrier spray were shown to significantly reduce mosquito catch numbers inside the treated barrier throughout the 8 week study, as compared to the control properties. The findings of these studies indicate that many factors, pertaining to both the insecticides used and to the environment in which they are applied, play a role in influencing the efficacy of a pyrethroid barrier treatment for the control of *Aedes* mosquitoes. Thus, it is important to gather relevant information before the application of a barrier spray treatment to design the most appropriate program for the situation.

Public Abstract

Mosquitoes in Virginia are capable of transmitting many different diseases to humans and livestock. Many different treatment options are available to protect humans from these populations of mosquitoes. Some of these options can be performed for a whole community, such as area-wide fogging or treatments from truck-mounted sprayers, while others are applied to properties individually, like mosquito misting systems or barrier sprays. Applying long-lasting insecticides to the edge of an area can help to protect the inside of the area from mosquitoes, and this is called barrier spraying. Barrier sprays, in particular, have become a popular choice for homeowners, and they are successful at limiting human exposure to local mosquito populations. The experiments conducted here looked at three different insecticides used in these treatments and compared them for their ability to inhibit and kill Asian tiger mosquito. Other factors that potentially influenced effectiveness were examined, such as the plant type, the length of time since the treatment was applied, and whether or not the mosquito had taken a blood meal. These experiments examined three commonly used pyrethroid insecticides to determine their efficacy against the local Asian tiger mosquito. Suspend Polyzone lasted a long time but did not produce sufficient mortality, Talstar Professional killed large numbers of mosquitoes, but for a short period, and Demand CS lasted a long time and showed a high mortality rate. These studies showed that the three different insecticides lasted on the plants for different amounts of time, but also that the insecticides needed different amounts of contact time to kill mosquitoes. This information is essential because understanding how a treatment loses effectiveness over time can help with deciding when retreatment is needed. It was also found that the different insecticides were affected by the plants that they were sprayed onto, meaning that certain insecticides did better when sprayed on specific plants. Results from these studies revealed that Asian tiger mosquitoes that had just fed on human blood were more likely to die from insecticide treatments. Thus, even if a mosquito should bite an infected person, barrier spray treatments of their property will lower the chances that the mosquito can spread the disease to other people.

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Attribution

Several colleagues aided in the writing and research presented here in the chapters of this dissertation. A brief description of their contributions is included here.

Chapter 2: The Effects of Plant Substrate, Insecticide, and Exposure Time on the Efficacy of Barrier Treatments Against *Aedes albopictus*.

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Chapter 3: The Effects of Plant Substrate, Insecticide, and Blood Meal Status on the Efficacy of Barrier Treatments Against *Aedes albopictus.*

As mentioned above, Dr. Carlyle Brewster, Dr. Nicola Gallagher, and Dr. Sally Paulson also contributed to this paper as they did for the previous paper.

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Chapter 1
Introduction and Literature Review

Mosquitoes (Culicidae) are a family of true flies (Diptera) consisting of over 3,000 species worldwide, and 176 species in the United States (AMCA 2020). Capable of spreading a wide variety of diseases, both to humans and other animals, these organisms have been a concern for humans for the entirety of our history. As the world began to become more connected through trade, the spread of vector mosquitoes into non-native ranges began to increase. The African-native mosquito *Aedes aegypti* (Linnaeus) was introduced into the Americas as a result of the slave trade, where it likely was present on ships carrying shipments of slaves to the New World. This mosquito introduced new jungle viruses like dengue and yellow fever into naïve populations, hence the adoption of its common name: the Yellow Fever mosquito (Arnebeck 2008). Yellow fever would experience outbreaks in areas such as Philadelphia, Pennsylvania in 1793 and Wilmington, North Carolina in 1862, and these outbreaks were possible because of the combination of available vector populations and socioeconomic factors (Moreno-Madriñán and Turell 2018).

With our current global-economy, worldwide trade and travel has never been so prevalent. A result of this has been an increase in the incidence of Zika (ZIKV), dengue (DEN), and Chikungunya (CHIK) viruses in the U.S. For example, in 2014, there were thousands of cases of CHIK in the U.S. and its territories, including both imported and locally-transmitted cases (CDC 2017). In a 2016 outbreak of ZIKV, more than 5,000 cases in the U.S. and nearly 37,000 cases in the territories were reported. Although most of the cases in the U.S. were imported, 224 cases were locally transmitted (CDC 2017). DEN is common throughout Central and South America and the Caribbean region. Although most DEN cases in the U.S. are travel related, between

2010-2017 there were locally acquired cases reported from Hawaii, Florida, Texas, and New York (Rivera et. al. 2020). ZIKV, DEN and CHIK are all anthroponotic viruses transmitted by *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), mosquitoes that are prevalent throughout much of the U.S. The increasing frequency of imported disease and the presence of vector mosquitoes in the southeast and mid-Atlantic region in the U.S. has led to increased interest from local governments, mosquito control districts, and individuals to manage mosquito populations.

1.1 History of Insecticides

The use of pesticides has existed for much of the history of human civilization. References date back to before 2000 BC in the Rig Veda, the classic book of Hinduism, which details the use of poisonous plants for control agricultural pest insects (Chopra et al 1949; Rao et al 2007). Later, chemicals such as arsenic, lead, mercury, and sulfur saw use in agricultural systems for pest control. Nicotine sulfate, an extract from tobacco leaves, was reported in 1690 as the first plant-derived insecticide, which was followed by the extraction of pyrethrins from pyrethrum flowers and rotenone from derris roots in the early 1800s (Tomizawa and Casida 2005; Brooker 2010).

Dichlorodiphenyltrichloroethane (DDT) was first synthesized in 1874 by the Austrian chemist Othmar Zeidler, and its insecticidal properties were first determined in 1939 by the Swiss chemist Paul Müller. The first widespread use of this compound was in World War II, where it was employed to control malaria, typhus, and dengue along warfronts when pyrethrum was unavailable. DDT was commercially available to farmers in 1945 for use in agriculture, and was significant in the eradication of malaria in Europe and North America (de Zulueta 1998;

CDC 2018). With the success of DDT, the World Health Organization proposed this eradication program in 1955, aiming to eradicate malaria in countries with low to moderate transmission rates all across the world (Mendis et al 2009). The program failed to be sustainable, however, and increased mosquito tolerance to the compound led to a resurgence of malaria in many areas, sometimes increasing above pre-treatment levels (Chapin and Wasserstrom 1981). In the end, this program by the WHO only succeeded in eradicating malaria in areas with high socio-economic status, well-organized healthcare systems, and lower/seasonal malaria transmission (Sadasivaiah et al. 2007). During this time, public concerns over the widespread use of DDT began to grow, culminating in the famous book *Silent Spring* by Rachel Carson in 1962. This book argued that the widespread use of pesticides like DDT was detrimental to both wildlife and the environment, and as a result was dangerous to human health (Carson 1962; Lear 2009). This led to the investigation of pesticide use in the United States by the Science Advisory Committee of the White House, and the committee reported that the claims put forth by Rachel Carson were vindicated, recommending a phasing out of persistent toxic pesticides (Greenberg 1963).

The Environmental Protection Agency (EPA) was formed by an executive order from President Nixon in 1970, with the goal of protecting human health and the environment. In 1972, the administrator of the EPA, William Ruckelshaus, announced the cancellation of DDT use for most applications (U.S EPA 1975). Later in the 1970s, the EPA would begin banning other organochlorine compounds, restricting which compounds were deemed appropriate for use in the field. Desired attributes of pesticides began to shift from long environmental persistence and effectiveness to compounds that degraded more rapidly under environmental conditions, but were highly effective when they were applied. This change would better allow for monitoring of environmental impacts caused by chemical treatments, and would potentially limit the amount of bioaccumulation that would occur in ecosystems. To fill the niche left by the absence of DDT, agriculture began to turn to alternative chemistries for pest control, and an old class of pesticide was revisited: the pyrethroids.

1.2 Pyrethroids

Pyrethroids are organic compounds that are synthetically derived to resemble the structure of natural pyrethrins (Soderlund et al. 2002). This class of pesticides represents a major percentage of products available for commercial pest control after the restrictions placed on the use of DDT and organochlorides/organophosphates. Pyrethroid compounds are split into two sub-classifications based on their chemical structures: type I pyrethroids lack a cyano moiety at the α-position, whereas type II pyrethroids have the α-cyano moiety present (Nasuti et al 2003). Type I pyrethroids exhibit “T syndrome”, which is characterized in rats by aggressive behavior, hyperexcitation, ataxia, whole-body tremor, convulsions, and in mammals by progressive paralysis, whereas type II pyrethroids induce a “type II choreoathetosis syndrome” or “CS syndrome” as an indicator of poisoning (Nasuti et al 2003), which is characterized in rats by hypersensitivity, choreoathetosis, tremors, rhythmic seizure, and profuse salivation without shedding (Verschoyle and Aldridge 1980; Narahashi 1985; Vijverberg and van den Bercken 1990). Type I poisoning involves the peripheral nervous system, and type II CS syndrome was associated with the involvement of the central nervous system (Lawrence and Casida 1982). This discovery regarding type II intoxication was determined to be correlated with the concentration of a type II pyrethroid in the brain, regardless of the mode of administration (Barnes and Verschoyle 1974; Ruzo et al. 1979).

Sodium channels are important for the mode of action for pyrethroids (Narahashi 1985; Ruigt et al. 1987; Soderlund and Bloomquist 1989; Vijverberg and van den Bercken 1990; Narahashi et al. 1992; Bloomquist 1996; Field et al. 2017), preventing the transition from an activated to an inactivated state (Davies et al. 2007). These modified sodium channels are unable to return to a closed state, which results in extended hyperexcitation as sodium continues to flow into the cell. This degree of modification is prolonged as a type II pyrethroid, as compared to a type I (Chinn and Narahashi 1986; Yamamoto et al. 1986; Holloway et al. 1989; Motomura and Narahashi 2000). Modulations of the immune system and endocrine system have also been reported as a result of pyrethroid exposure, as well as oxidative stress in rats (Righi et al. 2009; Yousef 2010; Ansari et al. 2012; Fetoui and Dgoura 2012; Khemiri et al. 2017). Models have been developed that predict the binding site for pyrethroid compounds, based on the Shaker rat-brain $K_v1.2$ structure (Long et al. 2005; O'Reilly et al. 2006). The model illustrated how the voltage-sensor domain (S1-S4 helices) was connected to the ion-conducting pore module (S5-S6 helices) by a helical S4-S5 linker. When modelled, the S4-S5 linker and the S5 and S6 helices of domain II formed a hydrophobic pocked with the S6 helix of domain III, and that this pocket faced the lipid bilayer. It was hypothesized that this pocket would be accessible to lipid-soluble insecticidal compounds (O'Reilly et al. 2006).

Pyrethroids have reduced efficacy in mammals, however, for a number of speculated reasons. One of which is due to differences in sodium channel sequences, where changes in the binding capacity of pyrethroids can occur from minor compositional changes (Vais et al. 2000; Soderlund 2012; Field et al. 2017). Acute symptoms have been reported from pyrethroid poisonings in humans, such as neurological and respiratory symptoms (Saillenfait et al. 2015), as well as associations between pyrethroid exposure and effects on male human reproduction (sperm sex ratio) and child neurodevelopment (Jurewicz et al. 2016; Watkins et al. 2016). Pyrethroids demonstrate differing efficacies for both mammals and insects, with the latter being over 2200 times more susceptible (Environmental Health Criteria 99 1990). The potency of neuronal toxins and detoxification differences between vertebrates and invertebrates contributes greatly to this drastic observation (Narahashi et al. 1995; Narahashi 1996; Song and Narahashi 1996). In addition, another explanation of the lower toxicity of pyrethroids in vertebrates is a result of the poor dermal absorption seen in mammals, and the metabolism of the parent compound into non-toxic metabolites (Bradbury and Coats 1989).

The use of pyrethroids does pose a risk to non-target organisms, especially aquatic and terrestrial arthopods (Elliot 1976, Smith and Stratton 1986, Inglesfield 1989, Antwi and Reddy 2015). It is therefore critical to abide by label guidelines when applying pyrethroids as an insecticide. General cautions, such as avoiding application to flowering foliage or near water, can help to mitigate the non-target effects of the application, but each insecticide label details this information as it pertains to the product in question.

1.2.1 Bifenthrin

Bifenthrin is a type I pyrethroid with the International Union of Pure and Applied Chemistry (IUPAC) name 2-Methyl-3-phenylphenyl)methyl (1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]- 2,2-dimethylcyclopropane-1-carboxylate. First registered for use in 1989, bifenthrin exists in over 600 products usable in the United States (Johnson et al 2010a; EPA 2010). The chemical structure of bifenthrin was discovered, developed, and commercialized by the FMC Corporation. Bifenthrin is permitted for use as an insecticide/miticide in a variety of indoor and outdoor residential and commercial applications, including indoor pet uses, food handling establishments, and livestock/agricultural commodities (EPA 2010a).

1.2.2 Deltamethrin

Deltamethrin is a type II pyrethroid with the IUPAC name [(S)-Cyano-(3-phenoxyphenyl)-methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate. One of the earliest discovered pyrethroids, it was described in 1974, entered commercial markets in 1978, and was first registered in the United States in 1994 (Johnson et al 2010b; EPA 2010). Deltamethrin is permitted for use on cotton, sorghum, artichokes, pears, vegetables, fruits, and tree nut crops for the control of a broad spectrum of pests, and it is also registered for use in residential and industrial applications for the control of cockroaches, pests of stored commodities, and other nuisance or destructive insects (EPA 2010).

1.2.3 Lambda-cyhalothrin

Lambda-cyhalothrin is a type II pyrethroid with the IUPAC name 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano(3-phenoxyphenyl)methyl cyclopropanecarboxylate. This compound is an equimolar mixture of four of the possible isomers of cyhalothrin, and was first registered for use in the United States in 1988 (Pesticide Fact Sheet Number 171: KARATE 1988). Lambda-cyhalothrin is permitted for use for the control of a wide range of insect pests on a variety of agricultural products, as well as used for the control of public health pests like mosquitoes and cockroaches in non-agricultural settings (EPA 2010).

1.3 *Aedes albopictus*

The Asian tiger mosquito, *Aedes albopictus* (Skuse), is an invasive vector mosquito in many parts of the world (Bonizzoni et al. 2013), and has been shown to be a competent vector for several viruses (Benedict et al. 2007). Adults can be identified by a single white dorsal band on the thorax, and by white banding on the legs. *Aedes albopictus* is native to Asia and was first shown to be identified in the continental United States in the mid-1980s. At the Port of Houston, *Ae. albopictus* mosquitoes were found in a shipment of used tires (Sprenger and Wuithiranyagool 1986), and spread rapidly to the east and north. As of 2017, this mosquito has been identified in 1,368 counties across 40 U.S. states (Hahn 2017), and expansions due to climate change, urbanization, and increased human movement will likely increase the spread of the organism further (Kraemer et al. 2019).

Laboratory studies of vector competence indicate that *Ae. albopictus* is capable of vectoring at least 22 known arboviruses (Gratz 2004; Schaffner et al 2013; Medlock et al 2015), but little is known about the role of *Ae. albopictus* in the event of an outbreak. Primarily an accessory vector, *Ae. albopictus* has functioned as the main vector in outbreaks of Chikungunya virus (Rezza et al. 2007; Tsetsarkin et al. 2007; de Lamballerie et al. 2008; Grandadam et al. 2011), but usually only facilitates a spread of a disease in the event of an outbreak. Adult females are aggressive daytime feeders, and feed on a wide variety of hosts (Sullivan et al. 1971), but have been found to be highly anthropophilic in urban and suburban settings (Faraji et al. 2014). The combination of high vector competence for arboviruses and the preference for vertebrate (especially human) bloodmeals makes *Ae. albopictus* a highly relevant public health concern, and this is the same conclusion reached by Faraji et al. (2014).

In addition to the public health concerns, *Ae. albopictus* also represents a nuisance organism for urban and suburban environments. Due to its aggressive biting nature, it is often the primary mosquito species eliciting complaints from the public in areas where it is present (Farajollahi 2009). Adaptations to using artificial containers for breeding sites also allow the species to persist in urban and suburban habitats (Barker et al.2003, Eisen and Moore 2013). Standard area-wide control methods, such as using spray trucks to administer insecticides (Mount et al. 1996), offer little control for this species since these methods are usually aimed to control crepuscular species and not diurnal species like *Ae. albopictus*. Determining suitable control methods for *Ae. albopictus* in urban and suburban settings has become increasingly important, both for public health reasons and as a quality of life measure.

1.4 Barrier Sprays

A variety of control techniques exist that utilize pyrethroids for pest control, but after the historical lessons learned from the liberal use of DDT, applications have been reduced for environmental concerns. One method for targeting adult mosquitoes is to treat plant foliage and other vegetation using residual pesticides for the reduction of a local population (Trout et al. 2007; Amoo et al. 2008; Doyle et al. 2009; Richards et al. 2017). The first application of insecticides to foliage with the intent of targeting resting mosquitoes was documented by Joseph Ginsburg in 1934 while at the New Jersey Agricultural Experiment Station (Ginsburg 1944). When applied to foliage in this way, to act as a barrier against entry by target insects, these treatments can reduce the amount of broad-spectrum chemicals put into the environment (Cilek 2008; Cilek and Hallmon 2006). In the United States, barrier treatments are typically applied every 21-30 days.

Barrier spray treatments are affected by a variety of factors including plant species (Doyle et al. 2009), type of equipment used (Farooq et al. 2010), environmental conditions (Allan et al. 2009), and repellency of the compound used (Manda et al. 2013), which is related to the level of exposure of the mosquito to the chemical. Common pyrethroids selected for use as barrier spray treatments include bifenthrin (Trout et al. 2007; VanDusen et al. 2016; Richards et al. 2017), deltamethrin (Cilek and Hallmon 2006; Richards et al. 2017), and lambda-cyhalothrin (Trout et al. 2007; Unlu et al. 2017). There are some non-pyrethroids chemicals that can be used as a residual barrier spray, such as botanical compounds, but these pesticides are often exempt from Federal toxicity testing/registration and little is known about the safety or effectiveness of these compounds.

Treatments of residual insecticides applied in this way have been successful in reducing local mosquito populations for residential areas (Qualls et al. 2012). This success has led to industry and commercial interest, due to 1) the effectiveness of the treatments applied, 2) economic benefits of applying targeted treatments to desired locations instead of broad applications, and 3) public requests for mosquito control with increased awareness of mosquito-borne illnesses (Stoops et al. 2019). Across the mid-Atlantic and Southeast regions of the U.S., residential barrier sprays have become a sought-after treatment option against local populations of *Ae. albopictus* mosquitoes. Barrier spray treatments have been shown to reduce local populations of *Ae. albopictus* (Trout et al. 2007, Li et al. 2010, Qualls et al. 2013, Fulcher et al. 2015, Marini et al. 2015), demonstrating that this treatment method is better able to control this mosquito than other treatment methods aimed at controlling other mosquito species. With the growth of the barrier treatment industry, additional investigations into the efficacy of different active ingredients and application methods needs to be conducted to preserve mosquito control tools for use in the future. Investigations of barrier treatment efficacies against mosquitoes of public health concern, as well as investigations of commonly-used insecticides, should help to provide mosquito control professionals with the knowledge necessary to protect the public from mosquito-borne diseases, even when facing new scenarios from a changing global climate.

1.5 Research Objectives

Although research has been done to evaluate the effectiveness of barrier spray treatments against various mosquito species, there is still much about the subject that is currently unknown. The experiments discussed here were performed to expand upon previous findings in the field and to contribute to the foundation of future research. The following questions were investigated for this goal: 1) Are pyrethroid barrier spray applications effective against *Aedes albopictus*, and if so, for how long, 2) Does the foliage to which a pyrethroid barrier treatment is applied impact the effectiveness against *Aedes albopictus*, 3) Does the blood meal status of an adult female *Aedes albopictus* influence its susceptibility to pyrethroid barrier treatments, and 4) Do barrier spray treatments reduce mosquito populations inside the desired area when applied in a suburban setting?

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Chapter 2

Effects of Plant Species, Insecticide, and Exposure Time on the Efficacy of Barrier Treatments Against *Aedes albopictus*

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2.1 Abstract

The effect of 5 plant species (*Miscanthus sinensis* [zebra grass], Buxus X [boxwood], Rhododendron X 'Chionoides' [rhododendron], *Thuja occidentalis* [arborvitae], and *Lonicera japonica* [Japanese honeysuckle]) and 2 rates of Demand® CS (AI lambda-cyhalothrin; 3.13 ml and 6.25 ml [AI]/L) on knockdown (1 h) and mortality (24 h) of adult female *Aedes albopictus* was evaluated over an 8-wk period. A significant difference in knockdown was observed between the 2 rates of Demand CS on the 5 plant species, with the highest proportion knockdown observed on zebra grass and rhododendron treated at the higher rate. Although mortality was ≥60 and 85% on the 5 plant species at the low and high rates of Demand CS, respectively, a significant difference was only observed on boxwood and Japanese honeysuckle. We also tested the residual toxicity of 3 barrier sprays (Demand CS [AI lambda-cyhalothrin], Talstar® Professional [bifenthrin], and Suspend® Polyzone® [AI deltamethrin]) and evaluated the efficacy of a short (5-min) exposure to the insecticides on knockdown and mortality of adults over time. Significantly higher knockdown was observed with Demand CS compared with Talstar Professional or Suspend Polyzone. Mean knockdown was ~98, 92, and 20% for Demand

CS, Talstar Professional, and Suspend Polyzone, respectively, at week 2, and ~98, 0, and 44%, respectively, 8 wk after treatments were applied. Adult mortality from the three insecticides, however, remained above 90% throughout the study. Lastly, the trends in proportion knockdown were similar for mosquitoes exposed for either 5 min or 24 h to the 3 insecticides. An overall decline in mortality over time, however, was observed for mosquitoes exposed for 5 min to the insecticides compared with mortality from 24-h exposure. The results suggest that Demand CS can be an effective barrier spray treatment against *Ae. albopictus* adults because efficacy is limited little by plant species, it has long residual toxicity, and it is effective against the insect after only 5 min of exposure.

KEY WORDS *Aedes albopictus*, plant species, lambda-cyhalothrin, bifenthrin, deltamethrin

2.2 Introduction

Aedes albopictus (Skuse) is an invasive vector mosquito in many parts of the world (Bonizzoni et al. 2013) and a competent vector for several viruses (Benedict et al. 2007). An aggressive human biter, *Ae. albopictus* is often the primary mosquito species eliciting complaints from the public in areas where it occurs (Farajollahi 2009). Because this species readily utilizes artificial containers for breeding, it is well adapted to suburban and urban habitats (Barker et al. 2003). In fact, *Ae. albopictus* has become the most important and sometimes the only mosquito vector in many urban areas (Bonizzoni et al. 2013). Once *Ae. albopictus* becomes established in an area it is very difficult to eradicate. Standard area-wide control methods such as the use of spray trucks to administer insecticides offer little control for this species since these methods are generally directed at crepuscular species and not diurnal species like *Ae. albopictus*. Because *Ae. albopictus* is a major biting pest in suburban yards and has the potential to transmit viruses such as Zika, homeowners in the United States have become increasingly concerned about its presence around their homes and are constantly in search of new methods of control.

A common recommendation for mosquito population control is reduction of larval development sites. However, mosquito breeding sites may be cryptic. Because many species of adult mosquitoes utilize plant foliage and other vegetation as resting and feeding sites, barrier treatments with residual pesticides can be an effective method for reducing populations (Trout et al. 2007; Amoo et al. 2008; Doyle et al. 2009; Richards et al. 2017). Barrier treatments applied only to vegetation help to decrease the use of broad-scale application of insecticides to the environment and prevent mosquitoes from entering untreated outdoor areas (Cilek and Hallmon 2006; Cilek 2008). Common barrier spray treatments have included lambda-cyhalothrin (Trout et al. 2007; Unlu et al. 2017), bifenthrin (Trout et al 2007; VanDusen et al. 2016; Richards et al. 2017), deltamethrin (Cilek and Hallmon 2006; Richards et al. 2017), and permethrin (Cilek and Hallmon 2006; Amoo et al. 2008). Treatments are typically applied every 21–30 d in the United States. Li et al. (2010) found that barrier applications using lambda-cyhalothrin lasted up to 9 wk and provided an 83–98% reduction in *Ae. albopictus* landing rate counts. In Queensland, Australia, Muzari et al. (2014) conducted weekly sweep-net collections in lambda-cyhalothrin treated and untreated areas and observed an 87–100% reduction in mosquito numbers for 9 wk post spray. The effectiveness of barrier spray treatments, however, can be impacted by several factors including plant species (Doyle et al. 2009), type of sprayer (Farooq et al. 2010), environmental conditions (Allan et al. 2009) and repellency of the chemical (Manda et al. 2013), which is related to the level of exposure of the mosquito to the chemical.

The goal of this study was to assess the effectiveness of barrier spray treatments applied under natural environmental conditions using laboratory bioassays. The specific objectives were to study the effect of plant species on the residual toxicity of Demand CS, to compare the residual toxicity of 3 commonly used barrier spray insecticides, Demand CS (lambda-cyhalothrin), Suspend Polyzone (deltamethrin), and Talstar Professional (bifenthrin), and to evaluate the effects of exposure time of the insecticide treatments against *Ae. albopictus.*

2.3 Materials and Methods

Effect of plant species

Five plant species common to residential landscapes in southwestern Virginia were selected for this study: *Miscanthus sinensis* (zebra grass), Buxus X (boxwood), Rhododendron X 'Chionoides' (rhododendron), *Thuja occidentalis* (arborvitae), and *Lonicera japonica* (Japanese honeysuckle). Plants of each species in 11.4-liter pots and of comparable size and age were purchased from the Christiansburg Garden Center (Christiansburg, VA, USA). Potted plants of each species were randomly selected and assigned to one of three spray treatment groups consisting of Demand CS (7.9% lambda-cyhalothrin; Syngenta Crop Protection, Greensboro, NC) at 3.13 ml [AI]/liter or 6.25 ml [AI]/liter, and water as a control. The treatments were applied to plants using a backpack mist blower (SR200, Stihl Corp., Virginia Beach, VA) to runoff ensuring that both the upper and lower surfaces of leaves were treated. After treatment, plants were grouped by treatment, planted evenly in a 2-m^2 plot under natural environmental conditions, and watered at the base as needed.

Leaves were sampled from plants in each treatment group between 7 September and 2 November 2015 with the first samples collected the day following treatment (i.e., week 0). Additional leaf samples were collected at weeks 1, 2, 3, 4, 6, and 8. At each sampling, 3 leaves were selected randomly from the plants in each treatment group avoiding new growth. The selected leaves were excised, placed into a 1-liter Double Zipper storage bags (Walmart, Christiansburg, VA) and transported immediately back to the laboratory. Nitrile gloves were worn during leaf removal with fresh gloves used to collect samples from each treatment group. The leaf samples were used in bioassays to test the effects of plant species on knockdown at 1 h and mortality at 24 h of *Ae. albopictus* (see **Leaf bioassays**).

Comparative residual toxicity of barrier sprays

Rhododendron X ‘Chionoides’ (*Rhododendron spp*.) plants in 11.4-liter pots were purchased from Fast Growing Tree Nursery (www.fast-growing-trees.com*; Fort Mill, SC. [accessed 6 October, 2017]).* Two plants were selected randomly and assigned to one of four treatment groups consisting of three insecticides and a water control. The spray treatments were Demand CS (lambda-cyhalothrin; AI 7.9%; Syngenta Crop Protection, Greensboro, NC, USA) at 6.25 ml/liter of water; Talstar Professional (bifenthrin; AI 7.9%; FMC Professional Solutions, Philadelphia, PA, USA) at 7.81 ml/liter of water, and Suspend Polyzone (deltamethrin; AI 4.75%; Bayer Crop Science, Research Triangle Park, NC) at 11.72 ml/liter of water. The three insecticides and water treatments were applied using a backpack mist blower (SR200, Stihl Corp., Virginia Beach, VA) to runoff ensuring that both the upper and lower surfaces of leaves were treated. Plants were grouped by treatment, planted evenly spaced in a 1-m^2 plot, and watered as needed. Leaves were sampled from treatment plants between 27 July and 19 October, 2016 with the first samples collected the day following treatment (i.e., week 0). Additional leaf samples were collected at weeks 1, 2, 4, 6, 8, 10, and 12. At each sampling, 5 leaves were selected randomly from the plants in each treatment group avoiding new growth. Selected leaves were processed as described above (see **Effect of plant species**). The leaf samples were used in bioassays to test the residual toxicity of barrier spray treatments on knockdown at 1 h and mortality at 24 h on *Ae. albopictus* (see **Leaf bioassays**).

Effect of exposure time

Leaf samples for this study were collected in a similar manner as described earlier (see **Comparative residual toxicity of barrier sprays**). Mosquitoes in this experiment were exposed to the treated leaves in vials for 5 min after which the mosquitoes were removed, placed into 220-ml plastic containers (Corning, Inc. NY), and observed for knockdown and mortality at 1 and 24 h, respectively (see **Leaf bioassays**).

Leaf bioassays

Two to 3-day old adult female *Ae. albopictus* were obtained from the Virginia Tech Medical Entomology Lab colony. This colony was established from mosquitoes collected in Blacksburg, VA and have been maintained in the lab for approximately three years. The mosquitoes were reared in an insectary at 24°C, 80% RH, and a photoperiod of 16:8 (L:D) h using the procedures described by Munstermann and Wasmuth (1985).

To test the effect of plant species and residual toxicity of barrier spray treatments, 10 mosquitoes were added to a 7-dram borosilicate glass shell vial (Thomas Scientific, Swedesboro, NJ) containing a single leaf from a plant in each treatment group. Leaves were positioned so that both sides were accessible to the mosquitoes. Mesh netting was placed over the open end of each vial and secured with a rubber band; a wet paper towel was placed over the vial to prevent desiccation of the mosquitoes. Knockdown and mortality were evaluated after 1 h and 24 h, respectively.

To study the effect of exposure time on the toxicity of barrier spray treatments, the mosquitoes were exposed to treated leaves in the vials for 5 min after which they were transferred to sterile 220-ml plastic containers (Corning, Inc. NY) and covered with mesh netting. Knockdown and mortality were evaluated after 1 h and 24 h, respectively.

The vials and plastic containers with mosquitoes were stored in the insectary for observation. Mosquitoes were considered knocked-down if they were unable to fly within the vial or container, and were considered dead if they were unable to stand after slight agitation.

Environmental data

Data on rainfall (mm), temperature (°C), relative humidity, and light intensity (PAR µmol/m²s) were collected at a central location at the edge of plots containing the potted plants using an m50G Wireless Cellular Data Logger (Decagon Devices, Pullman, WA).

Statistical analysis

The effects of plant species on knockdown and mortality of adult female *Ae. albopictus* were determined using a linear mixed model for repeated measures ANOVA with an autoregressive covariance structure (Littell et al. 2000, Traver et al. 2018). In the model, plant species (arborvitae, boxwood, Japanese honeysuckle, rhododendron, and zebra grass), spray treatment (Demand CS [lambda-cyhalothrin] at 3.13 ml and 6.25 ml [AI]/liter, and water), sampling week (0, 1, 2, 3, 4, 6, and 8), and their 2- and 3-way interactions were the fixed effects factors. In the presence of a significant 3-way interaction, we analyzed one of the 2-way interactions at each level of the third factor, and repeated this process for each of the other two factors as suggested by Ott and Longnecker (2001). Data on the residual toxicity and exposure time of barrier spray treatments of mosquitoes exposed to treated rhododendron leaves were analyzed using a modified version of the linear mixed model for repeated measures ANOVA. Spray treatments (Demand CS [lambda-cyhalothrin], Suspend Polyzone [deltamethrin], Talstar Professional [bifenthrin], and water), sampling week (0, 1, 2, 4, 6, 8, 10, and 12), and their interaction were the fixed effects factors. Prior to each analysis, the response variable measurements, proportion knockdown and mortality, were tested for normality and transformed using an arcsin $\sqrt{y}$

transformation (Zar 2010). Post hoc multiple comparison tests were carried out with Tukey's HSD. All statistical analyses were performed using JMP Pro v13 (SAS, 2016) at a significance level of $\alpha = 0.05$.

2.4 Results

Effect of plant species

The analysis showed that there was a significant interaction of plant species × spray treatment ($F_{8,\,26.5} = 2.9337$, $P = 0.0174$, Fig. 2.1A) and spray treatment × sampling week ($F_{12,\,157.6} = 45.7643$, $P < 0.0001$, Fig. 2.2A) with respect to mosquito knockdown after 1 h. The highest proportion of knockdown (> 80%) was observed on zebra grass and rhododendron at the higher rate of Demand CS. A significant decline in 1-h knockdown was observed for the lower rate of Demand CS after week 0. However, knockdown remained relatively high up to week 4 at the higher rate after which it declined significantly (Fig. 2.2A).

A significant interaction of plant species × spray treatment ($F_{8,\,28.9} = 8.9627$, $P < 0.0001$, Fig. 2.1B) and spray treatment × sampling week ($F_{12,\,153.0} = 12.4952$, $P < 0.0001$, Fig. 2.2B) was also observed for 24-h mortality. Post hoc analysis showed that the significant interaction of plant species × spray treatment resulted mainly from differences between the two rates of Demand CS on boxwood and Japanese honeysuckle (Fig. 2.1B) with higher mortality observed at the higher rate. A significant decline in 24-h mortality after week 2 occurred at both rates of Demand CS although mortality at the higher rate remained above 80% throughout the study (Fig. 2.2B).

Comparative residual toxicity of barrier spray insecticide bioassays

The results showed that there was a significant interaction of spray treatment × sampling week with respect to 1-h knockdown ($F_{21,\,92.8} = 14.9768$, $P < 0.0001$). Knockdown was >90% in the Talstar Professional and Demand CS treatments up to weeks 2 and 8, respectively (Fig. 2.3A). The proportion of mosquito knockdown in the Suspend Polyzone treatment varied between 10–70% for the first 10 wk of the study. Knockdown declined for all insecticide treatments after week 10 but remained above 60% for the Demand CS treatment.

No significant differences were observed among the Demand CS, Talstar Professional, and Suspend Polyzone treatments with respect to mean 24-h mortality ($P > 0.05$) with mortality in these treatments consistently ≥90%. Mortality in the water control treatment was significantly lower (mean = 8.25%) throughout the study compared with the three insecticide treatments ($F_{21, 88.9} = 3.7155$, $P < 0.0001$; Fig. 2.3B).

Short exposure time bioassays

Mean proportion 1-h knockdown after mosquitoes were exposed to the treated leaves for only 5 min (short exposure) varied significantly over time among the spray treatments ($F_{21, 46.3} = 11.0540$, $P < 0.0001$; Fig. 2.4A). Proportion knockdown was >75% in the Demand CS treatment for the first 8 wk of the study; knockdown in the Talstar Professional treatment was >80% up to week 2 after which a significant decline was observed. Knockdown in the Suspend Polyzone treatment varied but was <40% throughout the duration of the study.

The trends in 24-h mortality after a short exposure to treated leaves were similar to those observed for the 1-h knockdown bioassay with mean 24-h mortality after 5-min exposure varying significantly over time among the treatments ($F_{21, 48.0} = 6.8808$, $P < 0.0001$; Fig. 2.4B). Mortality in Talstar Professional treatment declined after week 2, but remained above 60% in the Demand CS treatment up to week 8. Relatively low mortality (<40%) was observed throughout the study in the Suspend Polyzone treatment, although mortality spiked to ~60% at week 8.

Environmental data

Mean temperature, mean percent relative humidity, and total precipitation during the study period in 2015 were 13.5 °C, 83%, and 311 mm, respectively (Table 2.1). Likewise, mean temperature, mean percent relative humidity, and total precipitation in 2016 were 20.3°C, 79%, and 43.3 mm, respectively (Table 2.1).

2.5 Discussion

Barrier spraying is the application of insecticide treatments to vegetation to reduce or prevent adult mosquitoes from entering the area of treated foliage, and has become a more appealing control option for managing pest mosquito populations (Anderson et al. 1991, Cilek and Hallmon 2006, Trout et al. 2007). To qualify as an effective barrier treatment, the insecticidal product must provide efficacy while exposed to environmental conditions, such as sunlight, rainfall, and temperature. The experiments conducted in this study looked to evaluate differences among different plant species as well as the variations among different exposures to insecticide treatments. The plants, commercial formulations, and application rates used in this study were chosen based on what applicators would use in the field.

In the experiment evaluating differences among the five plant species, the highest proportion knockdown (>80%) was observed on zebra grass and rhododendron at the higher rate of Demand CS (Fig. 2.1A). The higher rate of Demand CS had the highest 1-h knockdown of *Ae. albopictus*, regardless of the time after treatment. The lower concentration of Demand CS had an equivalent level of knockdown at the initial treatment, but was significantly less effective thereafter. Previous research found knockdown rates by pyrethroids to decline after only 1 wk (Anderson et al. 1991; Cilek and Hallmon 2006; Doyle et al. 2009). Relatively high (>60%) mortality was recorded on all plant species although the differences were observed between the low and high rates of Demand CS on boxwood and on Japanese honeysuckle (Fig. 2.1B). A significant decline in mortality occurred at both rates of Demand CS after week 2 across all plant species although mortality at the higher rate remained above 80% throughout the study (Fig. 2.2B). The reduction in both knockdown and mortality at weeks 3 and 4 could be the result of the torrential rainfall (220 mm) that occurred during the time period.

Plant species vary in their leaf abundance, arrangement of leaves, and chemical makeup of the cuticular wax coatings on the leaves. Any or all of these factors could play a role on affecting the residual capabilities of a pesticide treatment. In a 12-wk study by Cilek and Hallmon (2006), reduced efficacy was observed with deltamethrin treatments due to new growth on plants, which provided non-treated resting material for the mosquitoes. A study of 16 different species of plants by Chowdhury et al. (2001) found that the efficacy of residual insecticides on *Folsomia candida* (Willem) (Collembola), a springtail that is widely used for estimating the effects of pesticides, varied from plant to plant. Chowdhury et al. (2001) found no obvious pattern in terms of plant species and characteristics to predict the effect. Similar to Doyle et al. (2009), our study found there was an effect of plant species on the efficacy of treatments, particularly with respect to mortality. However, the differences were much more subtle compared with those reported by Doyle et al. (2009). One possible explanation for the reduced effect of plant species in our study may have been that the backpack mist blower used was more effective at covering leaf surfaces than the handheld pump used by Doyle et al. (2009). This experiment evaluated a microencapsulated formulation of Demand CS (AI lambda-cyhalothrin). The polymer wall of the microcap (Scher et al. 1998) may provide protection from degradation on hostile surfaces, yet still be biologically available to the insect, hence the minimal effect of plant species reported in this study.

In the experiment examining differences among insecticides, Demand CS showed that it could be used effectively as a barrier spray for *Ae. albopictus* over a 2-month period, which compared favorably to the finding of Li et. al. (2010). However more research is needed to determine if other lambda-cyhalothrin formulations can provide the same extended residual coverage or if this is unique to Demand CS. The differences between the pyrethroids evaluated were also most likely due to the active ingredients and proprietary formulations. Doyle et al. (2009) evaluated bifenthrin (Talstar®One) and observed a significant drop in knockdown after the first week, where the average knockdown (1-hr exposure) 14 days post-treatment after was ~2.88%. Results from the current study found that a 1 hr exposure to Talstar Professional-treated rhododendron leaves resulted in >90% knockdown at 14 days post-treatment. The increased suppression may be due to the back pack mist blower used to treat leaves in this study or differences in insecticide formulation. When comparing the 24-hour exposure mortality rates in this study, there was little difference in mortality among Demand CS, Suspend Polyzone, and Talstar Professional (Fig. 3B). Examining the results of the short-term exposure bioassays, however, revealed significant differences among the three pyrethroids. Suspend Polyzone was less than half as effective as Demand CS, and the efficacy of Talstar Professional dropped dramatically after 2 wk. This would indicate that Demand CS lasted approximately twice as long as Talstar Professional when used as a barrier spray, and that Suspend Polyzone, due to its low efficacy in the short-term exposure trials, may not provide significant levels of control in the field. Additional evaluations of this formulation (Suspend Polyzone), however, may be warranted. Richards et al. (2017) observed that deltamethrin (Suspend Polyzone) and bifenthrin (Bifen I/T) significantly reduced the abundance of *Psorophora columbiae* (Dyar and Knab) (Culicidae) adults; however, no other key species, including *Ae. albopictus*, experienced significant decreases in abundance between treatments. The authors did note this could be attributed, in part, to the low sample size during the mosquito season.

Mosquitoes exhibit distinct daily rhythms in flight activity, feeding, reproduction, and development (Rund et al. 2013), which makes it extremely unlikely that a mosquito will rest on a treated substrate for a full 24 h. The circadian rhythms of *Ae. albopictus* have been shown to be modulated by reproductive state and feeding status (Lima-Camara et al. 2014), and only mated, bloodfed females showed significant reduction in locomotion. As a result, the mortality assessment in the 5-min exposure trials, although rarely done in bioassays, may be more biologically meaningful than at the longer exposures. The most common evaluation of insecticide efficacy uses the full 24 h exposure timeframe, which may not accurately represent real-world conditions. Current WHO guidelines, while initially designed for evaluating insecticides used in bed netting against malaria vectors, does provide some precedent for short exposure periods. The initial screen and assessment of insecticide efficacy is conducted using a WHO cone test in which mosquitoes are exposed to treated material for just 3 min and mortality recorded a day later (WHO 2006).

This study provides additional support for the microencapsulated formulation of lambda-cyhalothrin for use as a barrier spray against *Ae. albopictus* (Trout et al. 2007). Furthermore, it shows that plant species does not appear to play a major role in the efficacy of the treatment. However, care should be taken to treat all of the target foliage evenly to ensure optimal coverage. Demand CS remained resistant to environmental degradation and outperformed the other treatments evaluated. The formulation can be differentiated due to the microencapsulation technology that protects the active ingredient from movement into or inactivation by the surface (Wege et al. 1999). Because of its increased residual activity, the frequency of reapplications may be reduced compared to other insecticides, resulting in benefits for the operator and environment.

Future studies could expand upon the list of pyrethroids tested, as well as compare different formulations of active ingredients. A cost-analysis could also be performed for these applications to determine the costs associated with treatments. Additionally, differences observed between 5-min and 24-h exposures were significant. These differences have potentially biologically relevant implications, indicating the importance of further investigation. Combined with mosquito surveillance data and an integrated pest management plan, barrier spray applications can be utilized as an effective option to suppress mosquito species, such as *Ae. albopictus*. However, while plant species had minimal impact on efficacy, the performance of an active ingredient and formulation can vary greatly.

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2.7 Figures

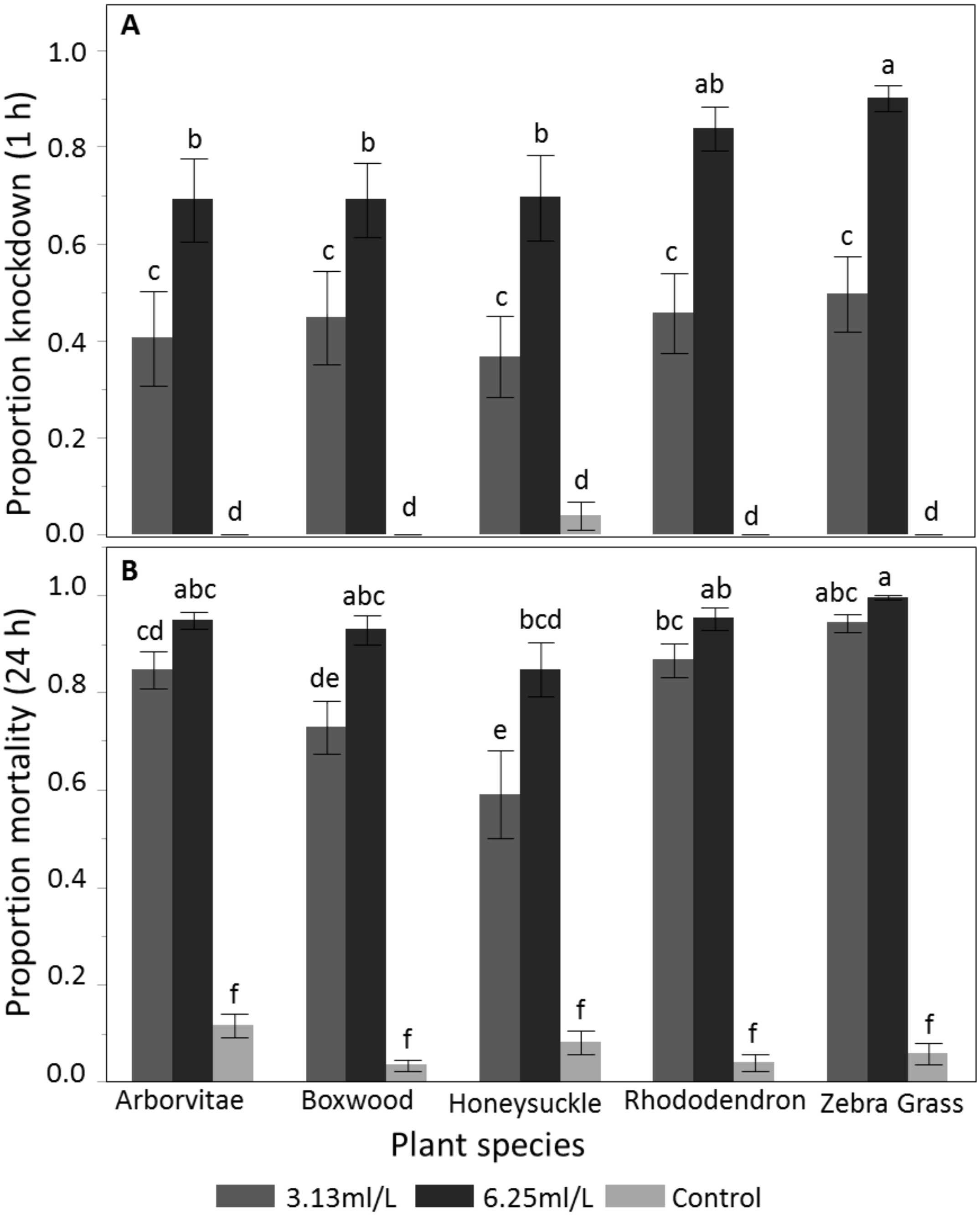

Figure 2.1 Proportion knockdown (Top; mean ± SE) and mortality (Bottom; mean ± SE) of *Ae. albopictus* to Demand CS (AI lambda-cyhalothrin) treated leaves by plant species. Knockdown evaluated at 1 h and mortality evaluated at 24 h. Means within each graph followed by the same letter are not significantly different. Tukeys HSD test (P > 0.05).

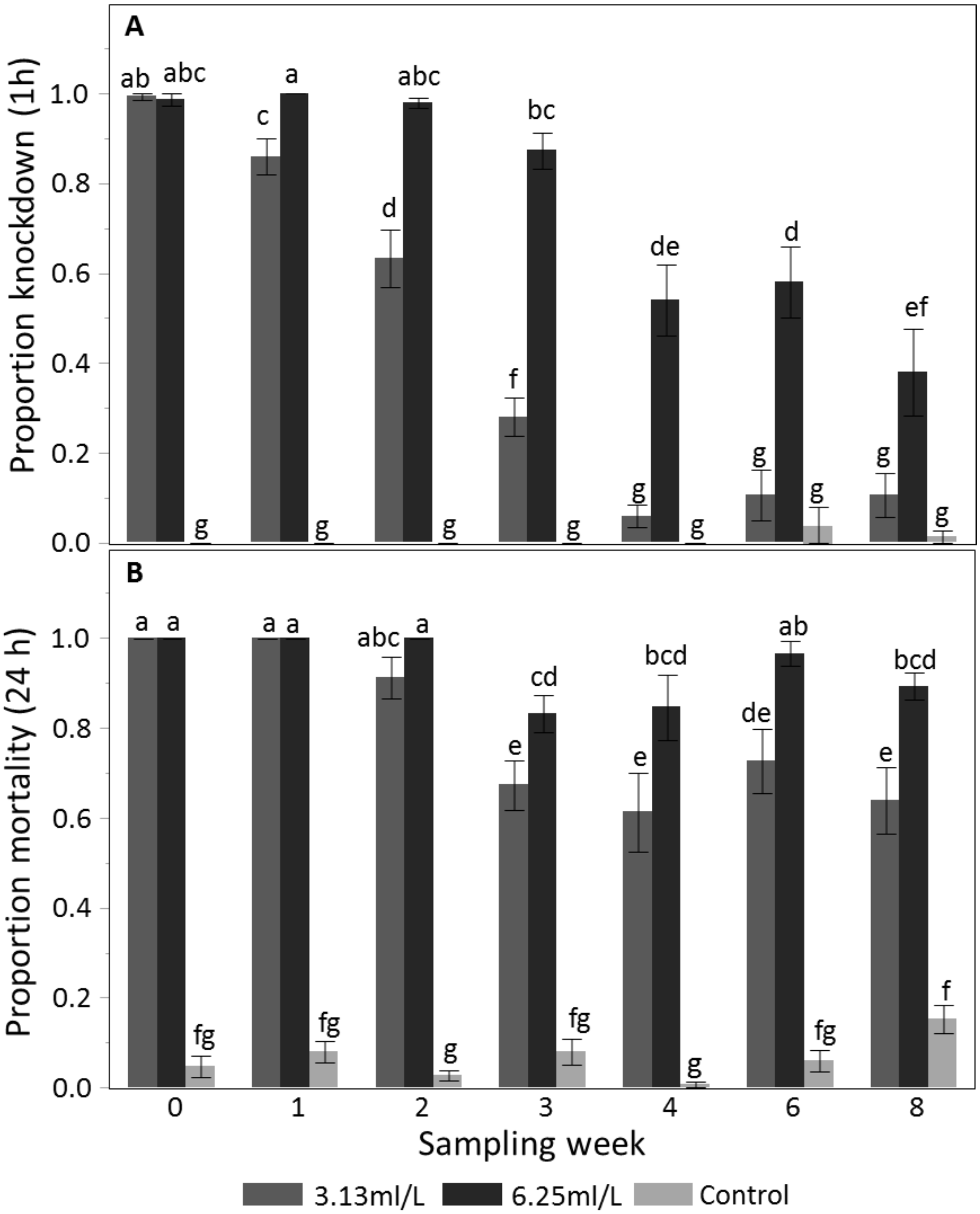

Figure 2.2 Proportion knockdown (Top; mean ± SE) and mortality (Bottom; mean ± SE) of *Ae. albopictus* after exposure to two treatments of Demand CS (AI lambda-cyhalothrin) for all plants over time. Knockdown evaluated at 1 h and mortality evaluated at 24 h. Means within each graph followed by the same letter are not significantly different. Tukeys HSD test ($P > 0.05$).

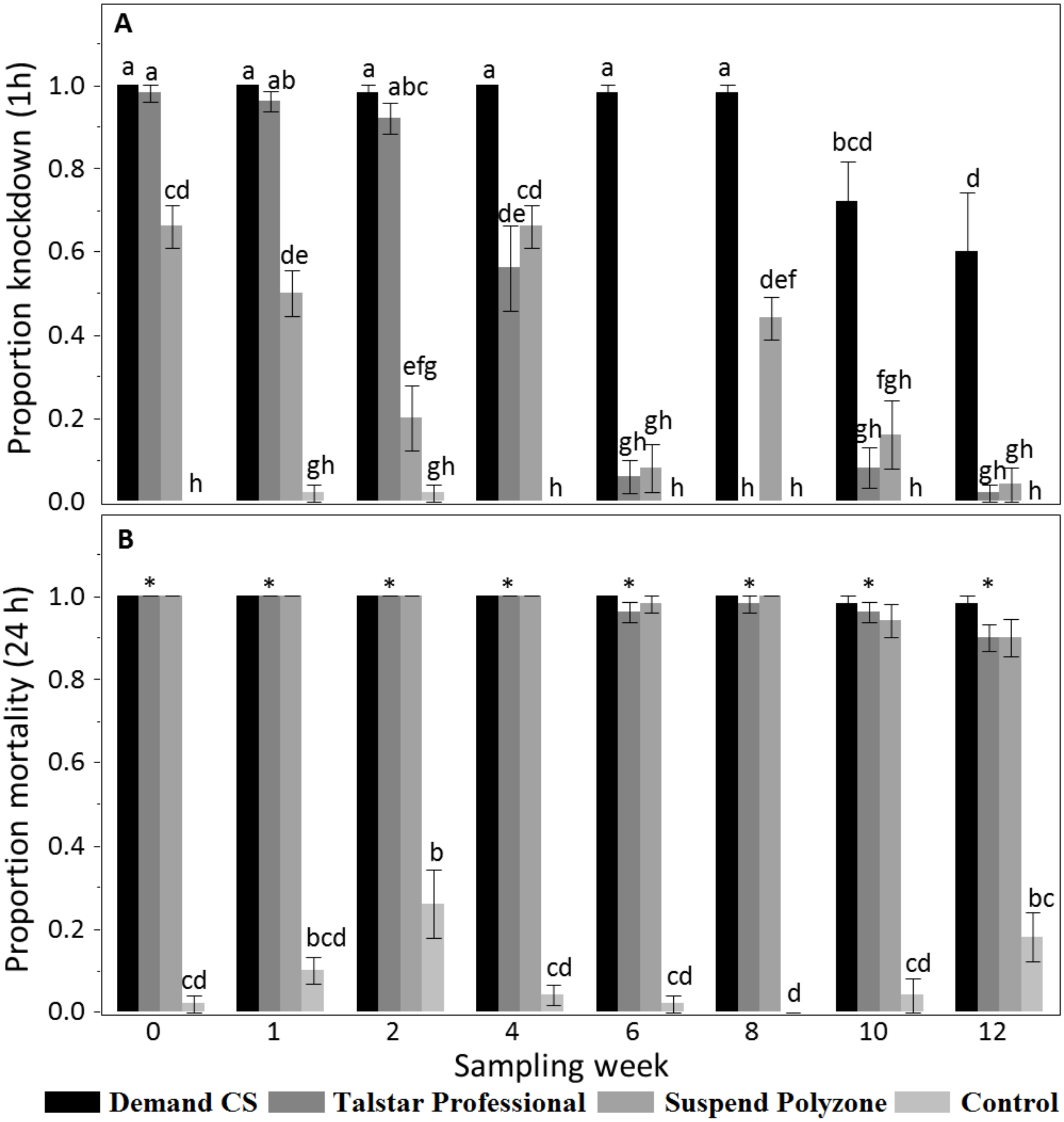

Figure 2.3 Proportion knockdown (Top; mean ± SE) and mortality (Bottom; mean ± SE) of *Ae. albopictus* to treated leaves over time. Knockdown evaluated at 1 h and mortality evaluated at 24 h. Means within each graph followed by the same letter are not significantly different. Groups marked with (*) are statistically similar. Tukeys HSD test (P > 0.05).

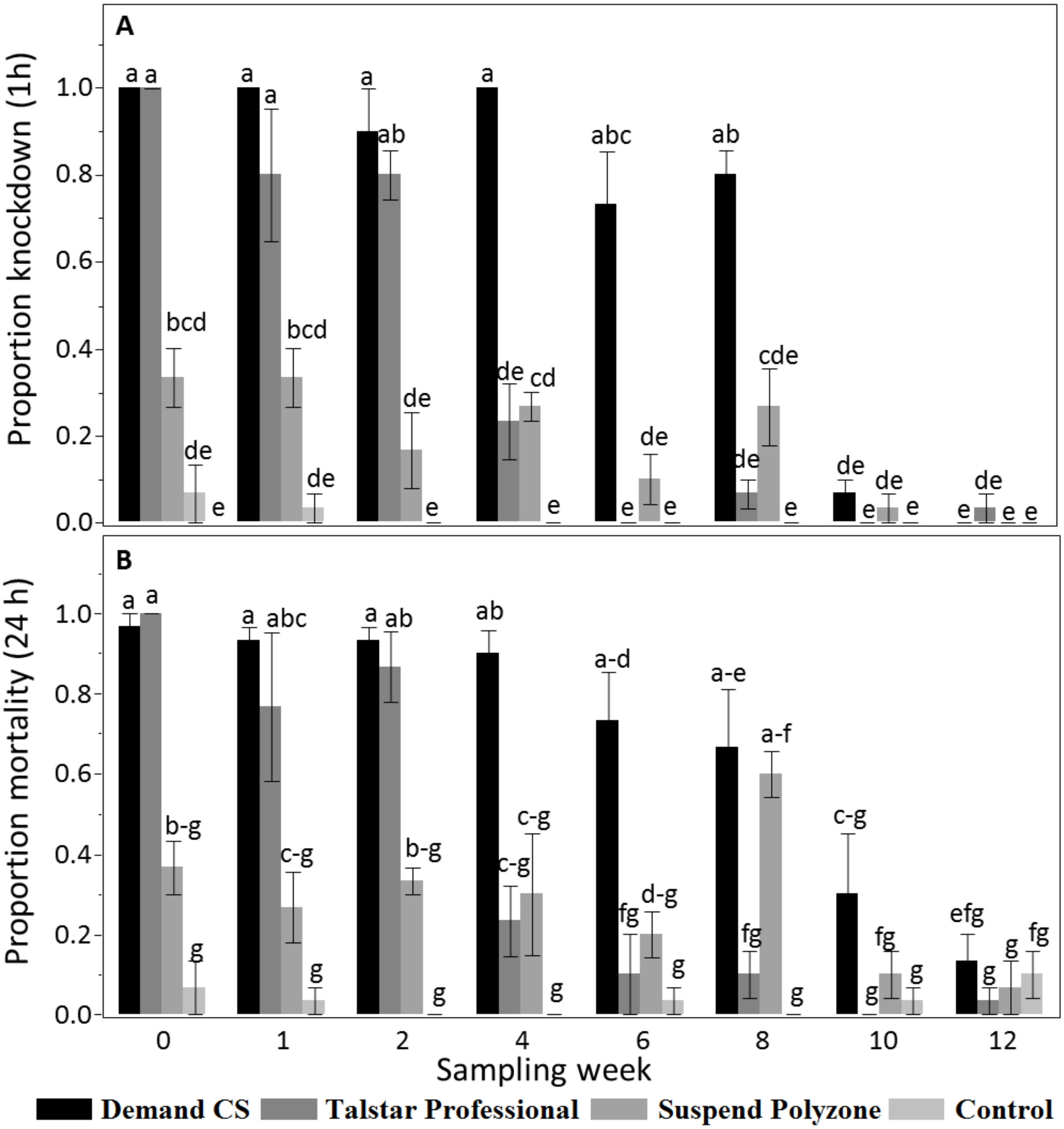

Figure 2.4 Proportion knockdown (Top; mean ± SE) and mortality (Bottom; mean ± SE) of *Ae. albopictus* after short exposure (5 min) to four treatments over time. Knockdown evaluated at 1 h and mortality evaluated at 24 h after exposure. Means within each graph followed by the same letter are not significantly different. Tukeys HSD test (P > 0.05).

Chapter 3

Effects of Plant Substrate, Insecticide, and Blood Meal Status on the Efficacy of Barrier Treatments Against *Aedes albopictus*

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3.1 Abstract

The effects of three plant species (*Cornus florida* (Linnaeus) [dogwood], Rhododendron X 'Chionoides' (Linnaeus) [rhododendron], and *Ilex opaca* (Aiton) [American Holly]), four insecticide treatments (Demand® CS [lambda-cyhalothrin] at 6.25 ml[AI]/liter; Talstar® Professional [bifenthrin] at 7.81 ml[AI]/liter, and Suspend® Polyzone® [deltamethrin] at 11.72 ml[AI]/liter, and water), and 2 physiological states (blood-fed and unfed) were evaluated for knockdown (1 h) and mortality (24 h) against female *Ae. albopictus* over an 8-week sampling period. Analyses determined that there was a significant interaction between the tested plant species and the insecticides evaluated. Significant differences were likewise observed between the insecticide treatments for unfed *Ae. albopictus* females, with Demand CS demonstrating the highest knockdown and mortality rates (from >90% to >10% at wk 8 and >95% to ~50% at wk 8, respectively), followed by Talstar Professional (from >75% to <10% at wk 2 and >90% to <10% at wk 2, respectively) and Suspend Polyzone (from >20% to <10% at wk 8 and >25% to >50% at wk 8, respectively). All treatments were no longer significant for knockdown or mortality at the end of the 8-wk timeframe. Significant differences were also observed between insecticide treatments for blood-fed *Ae. albopictus* females; Demand CS showed high knockdown and mortality rates (from 100% to ~50% at wk 8 and 100% to >60% at wk 8, respectively), Suspend Polyzone rates were similar to Demand CS (from >80% to ~50% at wk 8 and ~90% to >65% at wk 8, respectively), and both were followed by Talstar Professional (from 100% to <10% at wk 4 and 100% to <20% at wk 4, respectively). All tested pyrethroid sprays showed a significant increase in effectiveness against recently blood-fed *Ae. albopictus* females, as compared to the unfed females. These results suggest that Demand CS can be used as an effective barrier spray against *Ae. albopictus* adults due to the limited impact of target foliage, its long-term efficacy under environmental conditions, and its continued effectiveness regardless of the blood meal status of the target mosquito.

KEY WORDS *Aedes albopictus*, lambda-cyhalothrin, bifenthrin, deltamethrin, blood meal

3.2 Introduction

The Asian tiger mosquito, *Aedes albopictus* Skuse, is a worldwide nuisance pest capable of vectoring several viruses (Benedict et al. 2007; Kraemer et al. 2015). This highly invasive mosquito is a generalist feeder on mammals, but has been shown to aggressively take blood meals from humans in areas where humans are readily accessible (Gomes et al. 2003; Ponlawat and Harrington 2005; Delatte et al. 2009). Due to its preference for feeding on human hosts, *Ae. albopictus* often causes public complaints in areas of heavy infestation (Farajollahi 2009). *Aedes albopictus* has become a public health concern across the globe due to the expansion of its habitable range in conjunction with changing climates (Ponlawat and Harrington 2005; Delatte et al. 2009; Kamal et al. 2018). This species is especially difficult to control because it utilizes artificial containers as breeding sites, which can make removal difficult (Barker et al. 2003; Eisen and Moore 2013).

Control of mosquitoes such as *Ae. albopictus* can take many forms, such as reduction of larval breeding sites or large area sprays, but many key breeding sites for mosquitoes can be cryptic and difficult to properly treat. Another approach is to apply residual pesticides to resting substrates, which has been shown to reduce populations of mosquitoes (Trout et al. 2007; Amoo et al. 2008; Doyle et al. 2009; Richards et al. 2017; Richards et al. 2019). This application method reduces the amount of insecticidal products applied to the environment, which can aid in decreasing the widespread use of chemicals for pest control (Cilek and Hallmon 2006; Cilek 2008). Many active ingredients have been applied in this manner, including lambda-cyhalothrin (Unlu et al. 2017; McMillan et al. 2018), deltamethrin (Cilek and Hallmon 2006; Richards et al. 2017; McMillan et al. 2018), bifenthrin (VanDusen et al. 2016; Richards et al. 2017; McMillan et al. 2018), and permethrin (Cilek and Hallmon 2006; Amoo et al. 2008). Typical re-treatment times in the United States range from 21 – 30 days after the initial application when applied by pest control operators using specified label rates. Due to the variance of environmental exposure, many factors can influence the effectiveness of a barrier spray treatment, such as plant species (Doyle et al. 2009; McMillan et al. 2018), type of sprayer (Farooq et al. 2010) and environmental conditions (Allan et al. 2009). Both Doyle et al. (2009) and McMillan et al. (2018) observed significant impacts from plant species, but did not explicitly control for the surface area the mosquitoes were exposed to. Leaf surface area was therefore controlled in the bioassays of this study to more accurately observe differences in insecticide treatment efficacy due to the plant species.

Another factor that has been investigated is how a recently-ingested blood meal impacts the mosquito's susceptibility to chemical treatments (Hunt et al. 2005; Spillings et al. 2008; Rajatileka et al. 2011; Oliver and Brooke 2014; Machani et al. 2019), as well as how the blood meal impacts the biochemistry of the mosquito (Benoit et al. 2011; Lahondère and Lazzari 2012). In *Anopheles gambiae* Giles, it was observed that female mosquitoes were less susceptible to pyrethroid exposure after being allowed to digest a blood meal (Machani et al. 2019), but similar information for *Ae. albopictus* is not available. Mosquitoes require suitable resting habitats to protect them from extreme temperature and desiccating conditions. This is especially critical for blood fed mosquitoes that require several days to digest the blood meal and mature a batch of eggs. Also, if the mosquito ingested an infected blood meal, an incubation period is necessary before they can transmit the disease pathogen. Because of their importance in population growth and possible disease transmission, recently blood-fed mosquitoes were used in this study to examine the impacts of a blood meal on barrier spray efficacy.

This study compares the knockdown and mortality rates of unfed and recently blood-fed adult *Ae. albopictus* females over an 8-wk period for 3 commonly-used pyrethroid barrier sprays (Demand CS [lambda-cyhalothrin], Suspend Polyzone [deltamethrin], and Talstar Professional [bifenthrin]) when applied to different plant species.

3.3 Materials and Methods

Plant species and treatment

Three plant species were selected for this study: *Cornus florida* L. (dogwood), Rhododendron X ‘Chionoides’ L. (rhododendron), and *Ilex opaca* Aiton (American Holly). Plants of each species of comparable size and age were purchased from the Fast Growing Tree Nursery (www.fast-growing-trees.com*; Fort Mill, SC, USA* [accessed 13 June 2019]). Two plants of each species were selected and assigned to one of four treatment groups: three insecticide treatments or water as the control. The spray treatments were the highest available label rates; Demand CS (lambda-cyhalothrin; AI 7.9%; Syngenta Crop Protection, Greensboro, NC, USA) at 6.25 ml/liter of water; Talstar Professional (bifenthrin; AI 7.9%; FMC Professional Solutions, Philadelphia, PA, USA) at 7.81 ml/liter of water, and Suspend Polyzone (deltamethrin; AI 4.75%; Bayer Crop Science, Research Triangle Park, NC, USA) at 11.72 ml/liter of water. Applications were administered to plants using a backpack mist blower (SR200, Stihl Corp., Virginia Beach, VA, USA) to the point of runoff with complete coverage of both upper and lower surfaces of leaves. Following the spray applications, plants were allowed to dry, grouped according to treatment, and planted evenly in a 2-m^2 plot. Plants were exposed to natural environmental conditions and watered at the base as needed. The test plot was located at Virginia Tech's Prices Fork Research Center, Blacksburg, VA, USA.

Mosquitoes

The adult female *Ae. albopictus* used in the study were from a colony established from mosquitoes locally collected in Blacksburg, VA, USA; this colony has been maintained by the Virginia Tech Medical Entomology lab for approximately 3 years. The mosquitoes were reared in an insectary at 24°C, 80% RH, and a photoperiod of 16:8 (L:D) h using the procedures described by Munstermann and Wasmuth (1985). Two to 3-day old mosquitoes were used for the unfed bioassays. For the blood-fed bioassays, two-week old mosquitoes were fed on a human host 15 min prior to use. To control for variation, the same researcher was used as the host for all the feedings, the feeding took place at the same time of day, and feeding was allowed to continue until repletion.

Leaf bioassays

Leaves were sampled from plants in each treatment group between 29 June and 24 August 2018 with the first samples collected the day following treatment (i.e., week 0). Additional leaf samples were collected at weeks 2, 4, 6, and 8. At each sampling, three leaves were selected from the plants in each treatment group, avoiding visible new growth. Leaves were selected from the same area on all the plants, and all sampling was done by the same individual to minimize variance between samples. The selected leaves were excised, placed into 1-liter Double Zipper storage bags (Walmart, Christiansburg, VA, USA) and transported immediately back to the laboratory. Nitrile gloves were worn during sampling and were changed between insecticide treatments to prevent cross-contamination. After transportation to the lab, excised leaves were trimmed and immediately used in bioassays, as described below.

Ten mosquitoes were added to a 7-dram borosilicate glass shell vial (Thomas Scientific, Swedesboro, NJ) containing a single leaf from a plant from one of the treatment groups and plugged with a cotton ball. Leaves were cut to fit into the glass shell vials and positioned so that mosquitoes could access both sides while in the vial. After a 5-min exposure, mosquitoes were transferred to sterile 220-ml plastic containers (Corning, Inc. NY) and covered with mesh netting. Knockdown and mortality were evaluated after 1-h and 24-h, respectively, and for both blood-fed and unfed females. Mosquitoes were considered knocked-down if they were unable to fly within the vial or container, and were considered dead if they were unable to stand after slight agitation.

Environmental data

Data on rainfall (mm), temperature (°C), and relative humidity (Table 3.1) were retrieved from Weather Underground (Strubles Mill station: KVABLACK63) (Weather Underground 2019). This weather station is located roughly 2 miles from the research plot.

Statistical analysis

Data on the residual toxicity of barrier spray treatments of mosquitoes exposed to treated leaves were analyzed using a linear mixed model for repeated measures ANOVA with a first-order autoregressive and random effect covariance structure (Littell et al. 2000, Feazel-Orr et al. 2016, McMillan et al. 2018). The model examined plant species (dogwood, rhododendron, and American holly), insecticide treatment (Demand CS, Suspend Polyzone, Talstar Professional, and water), mosquito blood meal status (unfed or blood-fed), sampling week (0, 2, 4, 6, and 8), and their interactions as the fixed effects factors. If the 4-way interaction of plant species × insecticide treatment × mosquito blood meal status × week was not significant, the model was rerun with this interaction effect excluded. In the presence of a significant 3-way interaction, we analyzed one of the 2-way interactions at each level of the third factor and repeated this process for each of the other two factors, as suggested by Ott and Longnecker (2001). Before each analysis, the response variable measurements, proportion knockdown and mortality, were tested for normality and transformed using a Box-Cox transformation (Osborne 2010). Shapiro–Wilk *W* test and/or the skewness and kurtosis values were used to judge the goodness-of-fit of the transformed data when compared to the normal distribution (Thode 2002; Zar 2010). Post hoc multiple comparison tests were carried out with Tukey's HSD or Student's t-test, where appropriate. All statistical analyses were performed using JMP Pro v14 (SAS 2019) at a significance level of $\alpha = 0.05$.

3.4 Results

Effect of plant species

In this study, analysis showed that there was a significant interaction between plant species and insecticide treatment for both knockdown ($F_{6,\,71.7} = 3.0925$, P = 0.001) and mortality ($F_{6,\,87.3} = 4.0274$, P = 0.001). Demand CS did not have significant differences in knockdown or mortality across the examined plant species, Talstar Professional varied significantly in observed mortality, and Suspend Polyzone varied significantly for both knockdown and mortality (Table

3.2). No significant 2-way interactions were observed between plant species and any other fixed effects, i.e., mosquito blood meal status and week.

Comparative residual toxicity of barrier spray insecticide bioassays

The results showed that there was a significant effect of insecticide treatment ($F_{3, 71.7}$ = 249.7380, $P < 0.0001$), mosquito blood meal status ($F_{1, 71.7}$ = 77.4261, $P < 0.0001$), week ($F_{4, 196.2}$ = 54.7980, $P < 0.0001$), the interaction of insecticide treatment × week ($F_{12, 212.8}$ = 19.2568, $P < 0.0001$; Figs. 3.1A and 3.2A, B) and the interaction of insecticide treatment × mosquito blood meal status ($F_{3, 71.7}$ = 23.5357, $P = 0.0001$; Fig. 3.3A) on mosquito knockdown at 1 hour. Overall, Demand CS had the highest proportion knockdown against blood-fed female *Ae. albopictus,* followed by Suspend Polyzone, Talstar Professional, and the control. For unfed mosquitoes, the Demand CS treatment also had the highest proportion knockdown, with similar knockdown observed with Suspend Polyzone and Talstar Professional.

However, the effects of the insecticides varied over time as shown through the interaction of insecticide treatment × week for blood-fed and unfed female *Ae. albopictus* (Fig. 3.2A, B). The efficacy of Demand CS declined steadily after week two and that of Talstar Professional decreased rapidly after week zero. Although the efficacy of Suspend Polyzone was initially lower than the other two insecticides, the proportion knockdown remained relatively steady throughout the study. At week eight, there was no significant difference in knockdown between Demand CS and Suspend Polyzone. After week two, knockdown from Talstar Professional was equivalent to that of the control (Fig. 3.1A).

With regards to mosquito mortality, significant effects were observed for insecticide treatment ($F_{3, 87.3}$ = 416.2152, $P < 0.0001$), mosquito blood meal status ($F_{1, 87.3}$ = 113.2793, $P < 0.0001$), week ($F_{4, 183.6}$ = 67.0994, $P < 0.0001$), the interaction of insecticide treatment × week

($F_{12, 197.6} = 32.4952$, $P < 0.0001$; Figs. 3.1B and 2C,D) and the interaction of insecticide treatment × blood meal status ($F_{3, 87.3} = 15.4379$, $P = 0.0001$; Fig. 3.3B). Trends in mortality were similar to those observed for knockdown with respect to each of the insecticide treatments. Mortality declined steadily from 100% to ~55% by week eight for Demand CS, remained between ~55% and ~70% for Suspend Polyzone, and dropped drastically from ~100% to the level of the control after week two for Talstar Professional (Fig. 3.1B).

Effect of blood meal

Blood meal status had a significant effect on the mosquito knockdown observed, with blood-fed mosquitoes being significantly more susceptible when exposed to insecticide treatments (Fig. 3A). Although there were higher knockdown numbers recorded for all insecticide treatments, Suspend Polyzone showed the greatest difference in knockdown between unfed and blood-fed mosquitoes. Unfed mosquitoes showed the greatest proportion knockdown from Demand CS exposure, followed by Suspend Polyzone, and then Talstar Professional (Talstar Professional was still significant from the controls). Demand CS also had the highest observed mosquito knockdown against blood-fed mosquitoes, with Suspend Polyzone showing comparable knockdown proportions and Talstar Professional being significantly less effective than both (but again still significant from the controls). Notably, blood-fed mosquitoes exposed to Talstar Professional experienced significantly lower proportion knockdown than unfed mosquitoes exposed to Demand CS, and were statistically similar to unfed mosquitoes exposed to Suspend Polyzone (Fig. 3.3A).

Blood meal status also had significant effect on observed mosquito mortality, again with blood-fed mosquitoes being significantly more susceptible to insecticide treatments than unfed mosquitoes (Fig. 3.3B). Demand CS and Suspend Polyzone again demonstrated similar mortality rates against blood-fed mosquitoes, but Demand CS caused significantly more mortality against unfed mosquitoes. Talstar Professional induced significantly less mortality than either of the other active ingredients, but again was still significant from the controls. Talstar Professional caused lower mortality rates against blood-fed mosquitoes than Suspend Polyzone did against unfed mosquitoes (Fig. 3.3B).

Environmental data

Mean temperature, mean percent relative humidity, and total precipitation during the study period were 22.4 °C, 80%, and 156.7 mm, respectively (Table 3.1). This temperature is consistent with the historical average temperature for this time period (~22.5°C) and is representative of typical average rainfall in the area (~152 mm), according to climate averages for the Blacksburg, VA area (weatherspark.com 2020).

3.5 Discussion

Barrier sprays have become a popular control option for mosquitoes in the United States, and using residual insecticides in this way has been shown to reduce or prevent adult mosquito pressure from the surrounding environment (Anderson et al. 1991; Cilek and Hallmon 2006; Trout et al. 2007). These residual compounds must remain effective despite environmental complications, such as temperature changes, sunlight degradation, and varying levels of moisture. The best compounds can withstand these changing conditions for long periods, and thus remain biologically available and effective for that timeframe, resulting in less frequent re-treatment requirements. This study was designed to examine how differences in plant species, insecticide formulations, and mosquito blood meal statuses might interact to influence the effectiveness of a barrier spray treatment against *Ae. albopictus*. The application rates and the commercial formulations used in this study were chosen based on typical options for applicators in the field.

Previous research suggests that the species of a plant has a significant impact on the efficacy of a barrier spray treatment (Doyle et al. 2009; McMillan et al. 2018). McMillan et al. (2018) suggested that this finding could have been related to differences in available surface area. In this study, we controlled for available surface area by trimming leaves to a uniform size before testing, and still found significant differences in efficacy between the insecticide treatments applied to the different plant species (Table 3.2). It was observed that the interactive effects between plant species and insecticide treatment were significant, with Suspend Polyzone showing significant differences in knockdown and mortality based on the plant species and Talstar Professional showing significant differences in mortality. Demand CS did not show any significant differences in either knockdown or mortality based on the plant species to which it was applied (Table 3.2). Chowdhury et al. (2001) conducted a study of residual pyrethroid effectiveness against *Folsomia candida* (Willem), a collembola commonly used to evaluate pyrethroid efficacy, and examined treatments applied to 16 plant species. Their study determined that a clear pattern for plant impact on residual effectiveness of pyrethroid treatments was not obvious, but also mentioned that observed differences may have been linked to varying levels of exposure (Chowdhury et al. 2001). Therefore, with the findings of this study and those of Chowdhury et al. (2001), it has been shown that plant species can have an impact on the residual effectiveness of pyrethroid treatments, but additional work on the topic needs to be conducted. Investigation into the influence of leaf cuticular wax may help to explain the differences in effectiveness observed from this and previous studies.

Significant differences in residual effectiveness between the three insecticide treatments were observed in this study, with the longest effect lasting eight weeks (Fig. 3.1). This residual effectiveness is similar to previous research on the topic (Li et al. 2010; McMillan et al. 2018) and showed that Demand CS can be used effectively as a barrier spray for approximately two months. Additional research on lambda-cyhalothrin could determine if this result is unique to the formulation of Demand CS or an aspect of the active ingredient. Differences observed between the pyrethroid insecticides tested in this study were most likely due to different active ingredients and formulations. Bifenthrin is a type I pyrethroid, whereas lambda-cyhalothrin and deltamethrin are both type II pyrethroids. Also, Talstar Professional (bifenthrin) is not a formulation that protects the active ingredient from environmental degradation, such as the microencapsulation present in Demand CS (lambda-cyhalothrin) or the microscopic polymer film from Suspend Polyzone (deltamethrin). These aspects could influence the effectiveness of the active ingredient when used as a residual barrier spray, and additional investigation of these effects would clarify these differences further. Doyle et al. (2009) reported that bifenthrin (Talstar®One) demonstrated a significant decrease in efficacy after one week, and our results support those observations. Against unfed female *Ae. Albopictus*, Demand CS showed the greatest knockdown/mortality, Suspend Polyzone showed low knockdown/mortality numbers throughout the study, Talstar Professional was similar to Demand CS on week zero for both knockdown and mortality, but was not significant for the remainder of the study. This information suggests that Demand CS lasts roughly three times as long as Talstar Professional when applied as a barrier spray in the field, and that Suspend Polyzone, due to its low efficacy throughout the study, may not provide significant levels of control in field applications. Additional observations of the deltamethrin formulation used in this study (Suspend Polyzone) may be warranted to corroborate the results described here.

The physiological state of *Ae. albopictus* has been shown to influence their circadian rhythms (Lima-Camara et al. 2014), with mated, blood-fed females demonstrated to have the only significant reduction in locomotion. This finding justifies a short exposure time in the leaf bioassays conducted in this study and the inclusion of blood-fed females explores the impact of two different physiological states. In all insecticide treatment groups, blood-fed female *Ae. albopictus* showed greater susceptibility to the treatments, represented as both higher knockdown and mortality numbers (Fig. 3.3). Knockdown measurements for Demand CS declined steadily from ~100% at week four down to ~50% at week eight, Talstar Professional knockdown dropped sharply from ~100% at week zero down to <10% at week four and showed no significant effect afterward, and Suspend Polyzone maintained between 50% and 80% knockdown for the duration of the study (Fig. 3.2B). For mortality, Demand CS again declined steadily from ~100% at week four down to ~50% at week eight, Talstar Professional dropped sharply from 100% at week zero down to <20% at week four and no longer effective from week four onwards, and Suspend Polyzone declined slightly from ~90% at week zero down to >60% at week 8 (Fig. 3.2D). Results from Doyle et al. (2009) showed that recently blood-fed *Ae. albopictus* were susceptible to TalstarOne (AI bifenthrin 7.9%) residual treatments for less than 14 days, using an exposure time of one hour, and those findings are expanded upon by the findings of this experiment.

Differences in observed susceptibility of *Ae. albopictus* females to various pyrethroid barrier sprays may be linked to the different stressors the mosquito experiences during the digestion of its blood meal. All three of the examined pyrethroids experienced a significant increase in knockdown and mortality against blood-fed *Ae. albopictus* females, as compared to unfed females (Fig. 3.3). Both Demand CS and Talstar Professional showed roughly a two-week extension in efficacy for knockdown and mortality before they began to lose effectiveness on blood-fed females, and Suspend Polyzone showed up to 300% increases in knockdown and mortality rates throughout the study (Fig. 3.2). The prolonged increase in Suspend Polyzone effectiveness against blood-fed *Ae. albopictus* females was significantly different from the other pyrethroids examined, and the knockdown and mortality rates for Suspend Polyzone were similar to Demand CS for the blood-fed trials (Fig. 3.3). It remains to be seen if this extended increase is unique to deltamethrin or if there are other aspects of the formulation that are producing this result. Additional investigation of how the presence of a blood meal increases the susceptibility of a female mosquito to insecticide treatments should be conducted to better understand this mechanism. Research understanding stress associated with digestion of a blood meal has been conducted for mosquitoes, but little information is available about the interplay between a insecticide treatment and a recently-taken blood meal. Blood-fed *Ae. aegypti* that had been allowed to digest blood meals had been shown to be less susceptible to ultra-low volume aerosol treatments of synergized resmethrin, but the earliest time those mosquitoes were observed was 24-h post-feeding (Reiter et al. 1990). Barrier spray treatments are primarily implemented to prevent mosquitoes from feeding inside the treated area and therefore should aim to be reliably effective on unfed mosquitoes in addition to recently blood-fed ones.

The results of this study provide further support for the use of a microencapsulated formulation of lambda-cyhalothrin as a barrier spray for the control of *Ae. albopictus* (Trout et al. 2007; McMillan et al. 2018). Also, the effectiveness of pyrethroids as barrier sprays does appear to be affected by the target foliage, thus care should be taken to ensure all surfaces are evenly coated. Barrier spray treatments using Demand CS remained effective throughout the 8-

wk study, demonstrating resistance to environmental degradation. The microencapsulation technology used in this formulation of lambda-cyhalothrin protects the active ingredient from degradation by the environment (Wege et al. 1999) and is a probable reason for the longevity of the treatment. Demand CS treatments outperformed the other insecticide treatments in this study, regardless of plant substrate or blood meal status, but Suspend Polyzone efficacy was equivalent in the trials using blood-fed mosquitoes. Due to this, applications utilizing Demand CS may require less frequent reapplications, resulting in lower costs for applicators and less product applied into the environment. Using a conservative mosquito season length of three months, Demand CS applied as a barrier spray could reduce the number of treatments required for population control from three total treatments down to only two, based on reapplication requirements of four weeks and six weeks, respectively. This 50% reduction in reapplication requirements could enable pest management companies to focus efforts on treating new areas instead of retreating previous areas, and would reduce the overall volume of insecticidal products applied to the environment. When used in conjunction with mosquito surveillance data and an integrated pest management practice, barrier sprays can serve as an additional treatment option for suppressing mosquito species like *Ae. albopictus*. When choosing to apply a pyrethroid in this way, however, performance can be influenced by the active ingredient and formulation chosen.

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3.7 Figures and Tables

Week #	**Rainfall (mm)**	**Humidity (RH)**	**Mean Temp (^{0}C)**
1	0.00	0.77	23.5
2	6.35	0.79	23.0
3	0.00	0.72	22.7
4	32.00	0.77	22
5	13.97	0.83	21.4
6	64.77	0.88	21.8
7	39.62	0.85	22.2
8	N/A	N/A	N/A

Table 3.1 Weekly rainfall (mm), relative humidity and mean temperature in Blacksburg, VA from June 29th to August 24th, 2018.

Insecticide treatment	**Plant species**	**Proportion knockdown (mean ± SE)**	**Proportion mortality (mean ± SE)**
Demand CS	Dogwood	0.68 ± 0.10 a	0.73 ± 0.09 a
Demand CS	American Holly	0.63 ± 0.10 a	0.72 ± 0.09 ab
Demand CS	Rhododendron	0.66 ± 0.09 a	0.70 ± 0.09 ab
Suspend Polyzone	Dogwood	0.40 ± 0.10 bc	0.56 ± 0.09 b
Suspend Polyzone	American Holly	0.51 ± 0.10 ab	0.69 ± 0.09 a
Suspend Polyzone	Rhododendron	0.36 ± 0.09 c	0.57 ± 0.09 ab
Talstar Professional	Dogwood	0.28 ± 0.10 d	0.35 ± 0.09 c
Talstar Professional	American Holly	0.24 ± 0.10 d	0.27 ± 0.09 d
Talstar Professional	Rhododendron	0.27 ± 0.09 d	0.31 ± 0.09 cd

Table 3.2 Proportion knockdown and mortality (mean ± SE) of *Ae. albopictus* for each plant species over 8 weeks, sorted by insecticide treatment. Exposure time of 5 minutes. Knockdown and mortality evaluated at 1 h and 24 h, respectively. Means within each column followed by the same letter are not significantly different. Controls not shown due to no significance. Tukey's HSD (*P* > 0.05).

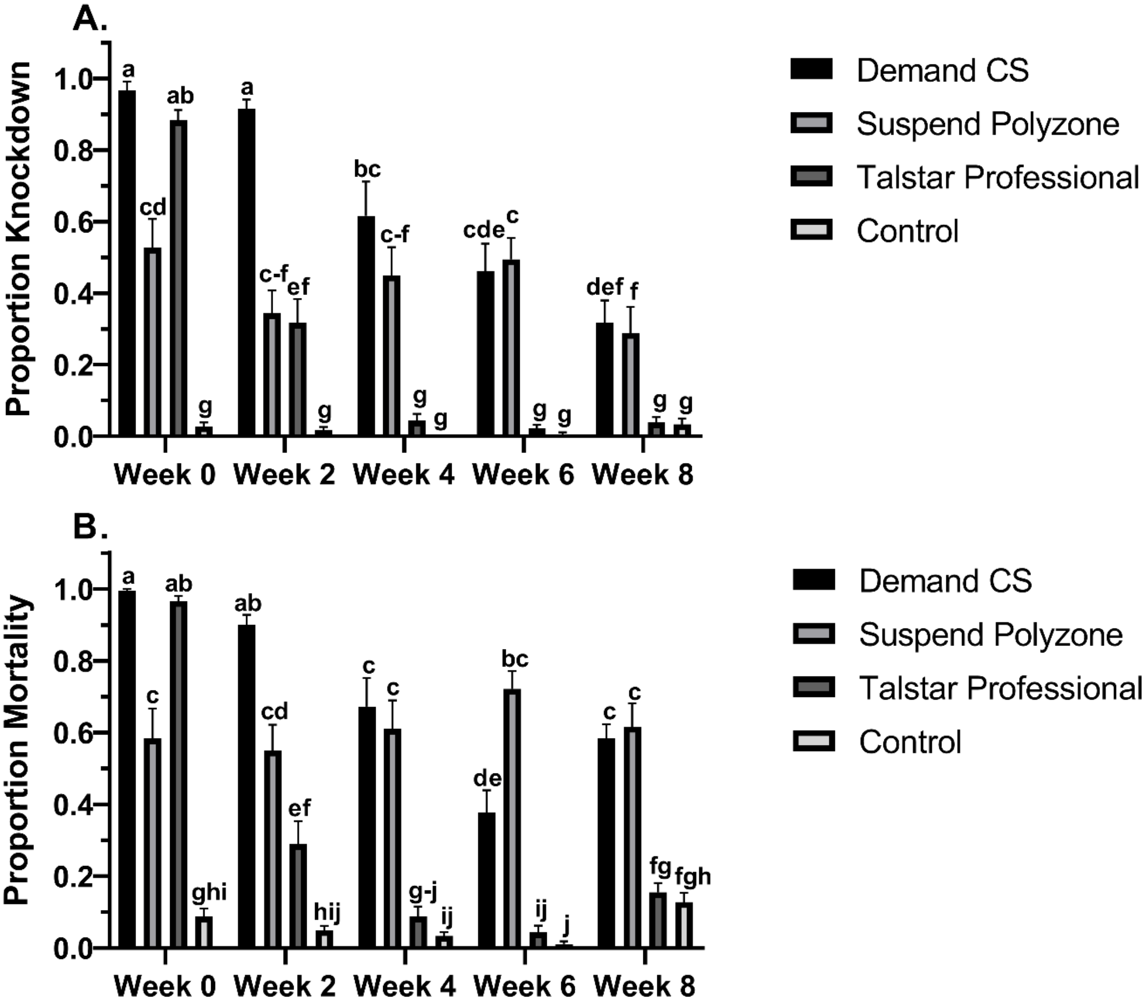

Figure 3.1 Proportion knockdown (A; mean ± SE) and mortality (B; mean ± SE) of *Ae. albopictus* by insecticide treatment over time. Exposure time of 5 minutes. Knockdown and mortality evaluated at 1h and 24h, respectively. Means within each graph followed by the same letter are not significantly different. Tukey’s HSD test (P > 0.05).

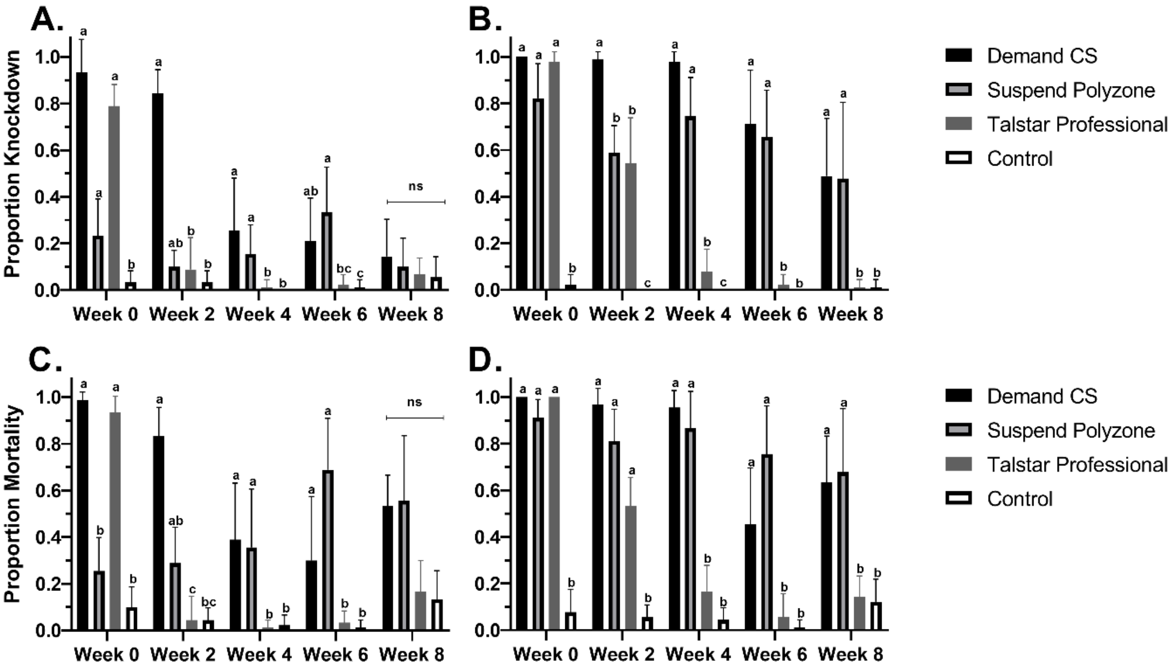

Figure 3.2 Proportion knockdown (Top; mean ± SE) and mortality (Bottom; mean ± SE) of unfed (A, C) and blood-fed (B, D) *Ae. albopictus* over time. Exposure time of 5 minutes. Knockdown and mortality evaluated at 1h and 24h, respectively. Means within each week followed by the same letter are not significantly different. Tukey's HSD test (P > 0.05).

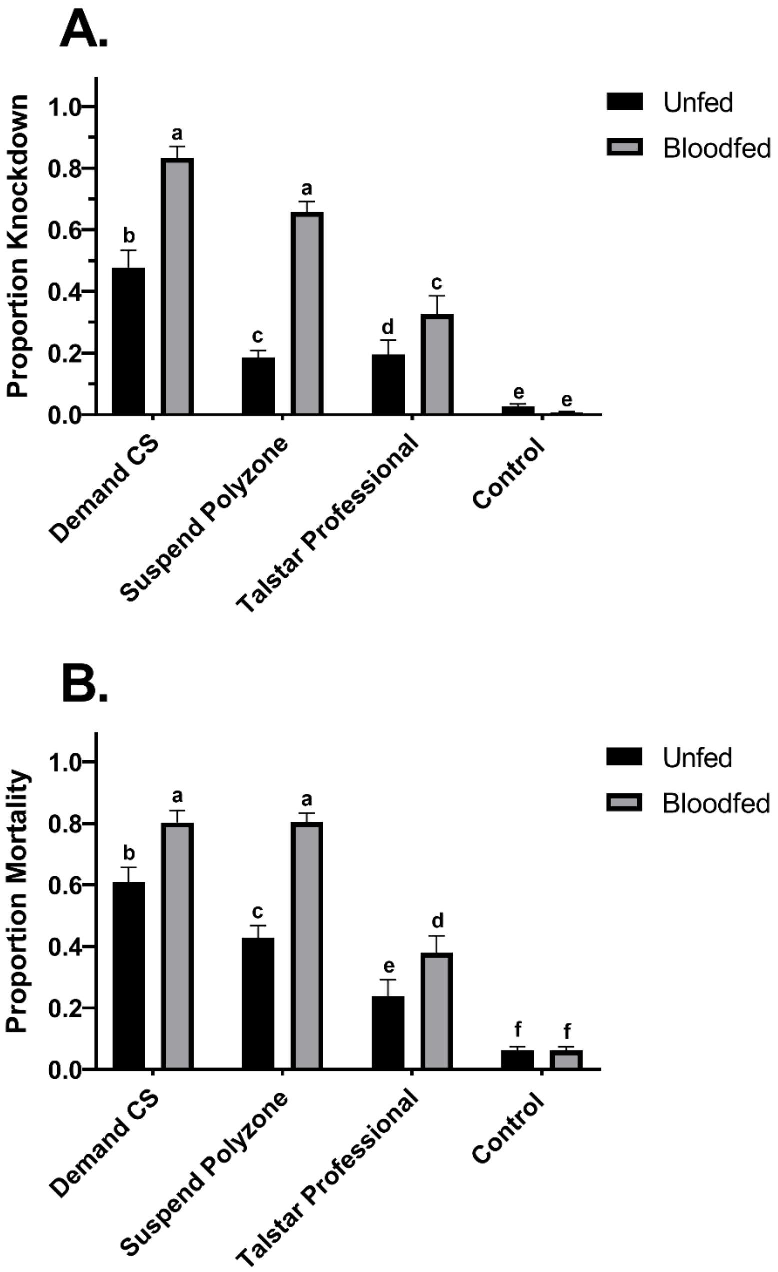

Figure 3.3 Proportion knockdown (A; mean ± SE) and mortality (B; mean ± SE) of *Ae. albopictus* by insecticide treatment, separated by physiological state. Exposure time of 5 minutes. Knockdown and mortality evaluated at 1h and 24h, respectively. Means within each graph followed by the same letter are not significantly different. Tukey's HSD test ($P > 0.05$).

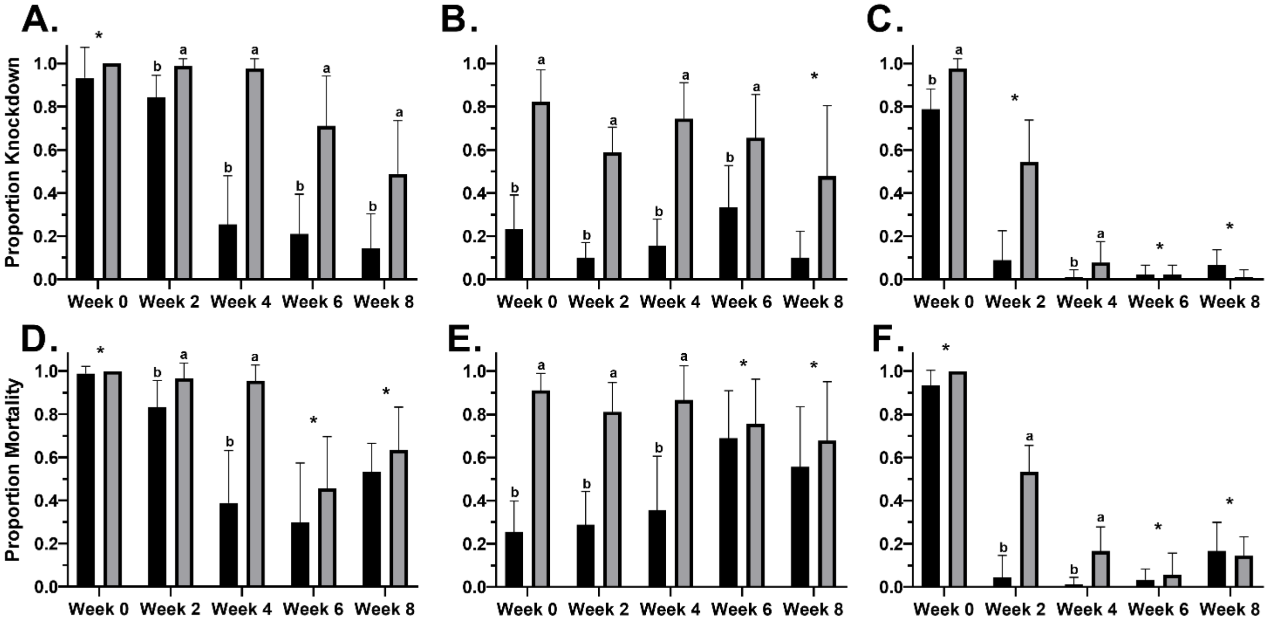

Figure 3.4 Proportion knockdown (Top; mean ± SE) and mortality (Bottom; mean ± SE) of unfed (black) and blood-fed (gray) *Ae. albopictus* by insecticide treatment over time. Insecticide treatments of Demand CS (A, D), Suspend Polyzone (B, E), and Talstar Professional (C, F). Exposure time of 5 minutes. Knockdown and mortality evaluated at 1h and 24h, respectively. Means within each week followed by the same letter are not significantly different. Controls not shown due to no significance. Tukey's HSD test (P > 0.05).

Chapter 4

Evaluation of Demand® CS Barrier Sprays for Mosquito Control in Southwestern Virginia

4.1 Abstract

This study evaluated the residual effectiveness of a Demand® CS barrier spray treatment when applied to suburban yards in Roanoke, Virginia. This evaluation took place from July 26th through October 3rd, 2018. Two BG-Sentinel traps baited with BG Lures were deployed weekly at each of the 12 participating locations for roughly 24 hours to determine the population of host-seeking mosquitoes present. Homeowner perceptions of treatment efficacy were obtained using questionnaires at the beginning, middle, and end of the study. Properties treated with Demand CS [A.I. lambda-cyhalothrin] experienced significantly lower mosquito catch numbers than did the water-treated control properties, though both populations experienced seasonal variation in mosquito abundance. This finding was supported by the survey responses from the participating homeowners, who were unaware of which treatment group they had been assigned. This result indicates that a Demand CS barrier spray treatment applied at the recommended label rate should reduce the pressure from the local population of adult mosquitoes for at least two months. Seasonal variation in local mosquito populations may still be present, but the magnitudes of populations local to the treatment would be significantly smaller than populations that had not received the Demand CS treatment. As part of an integrated pest management approach, barrier sprays can be used to reduce exposure to the local mosquito population by deterring mosquitoes from resting on treated substrates nearby. These results provide possible economic and environmental incentives for the use of barrier spray treatments by pest management companies for residential mosquito control.

KEY WORDS Lambda-cyhalothrin, residential, mosquito control, barrier sprays, *Aedes*

4.2 Introduction

Pest management companies in the United States serve an important role in the fight to protect the public from mosquito-borne diseases and nuisance mosquito populations. Area-wide treatment options are available to mosquito control districts, such as the use of truck-mounted sprayers to administer insecticides (Mount et al. 1996, Fulcher et al. 2015), but options for individual homeowners are more limited. An increasingly popular option available from commercial mosquito control companies is the application of an insecticide as a residual barrier spray, which has been shown to effectively reduce local populations of adult mosquitoes (Trout et al. 2007, Amoo et al. 2008, Doyle et al. 2009, Richards et al. 2017). Residual barrier treatments are applied to resting surfaces or vegetation where mosquitoes would sugar-feed (Fulcher et al. 2015), and have been shown to reduce the amount of broad-scale insecticides applied to the environment (Cilek and Hallmon 2006, Cilek 2008).

Many active ingredients and formulations are available for use as barrier sprays, such as lambda-cyhalothrin (Trout et al. 2007, Unlu et al. 2017, McMillan et al. 2018), bifenthrin (Trout et al. 2007, VanDusen et al. 2016, Richards et al. 2017, McMillan et al. 2018), deltamethrin (Cilek and Hallmon 2006, Richards et al. 2017, McMillan et al. 2018), and permethrin (Cilek and Hallmon 2006, Amoo et al. 2008). If re-treatments are required, applications of these residual insecticides typically occur every 21-30 days in the United States. External factors can influence the need for such re-treatment, such as the method of application (Farooq et al. 2010), the repellency of the chemical (Manda et al. 2013), the plant species to which treatment was applied (Doyle et al. 2009, McMillan et al. 2018), and the conditions of the environment (Allan et al. 2009).

The present study evaluated efficacy of Demand CS [AI lambda-cyhalothrin] for residential mosquito control in Roanoke, Virginia when applied as a residual barrier spray to suburban yards. The specific objectives were 1) to evaluate changes over time in local mosquito catch numbers pre- and post-application of a Demand CS barrier spray treatment and 2) to assess the perceptions of homeowners regarding the effectiveness of the treatment that had been applied to their residence.

4.3 Materials and Methods

Participants

Homeowners in the Roanoke area were targeted for recruitment based on interest expressed to the collaborating pest control operation (Bug Man Exterminating, T. Nininger, personal communication). Participants were recruited for voluntary involvement in the study if they were already in the process of purchasing a mosquito treatment from the collaborator, as this was a likely indicator of existing mosquito pressure. Residences, where the homeowner volunteered to participate, were screened one week before the beginning of the study for the baseline mosquito population at the location. Participants were provided the barrier spray treatment service free of charge and were given information regarding the treatments that were possible in the study. Participants were blinded to which treatment their property had received and were told beforehand that the study contained control properties.

Location

The study properties were located on a 300 km^2 plot of land north of the Roanoke River (Fig. 4.1) and the study was conducted between July 26th and October 3rd, 2018. Vegetation abundance at both treatment and control properties were determined to be similar from a visual inspection, and pre-treatment mosquito populations were collected to ensure comparable mosquito populations were present in all properties involved in the study.

Mosquitoes

Mosquitoes were sampled weekly from July 26th through October 3rd, 2018. The 12 participating locations were divided into two groups of 3 treatment and 3 control properties each. One group was sampled starting on Mondays; the second group was sampled starting on Tuesdays. Two BG-Sentinel traps (BioGents, Regensburg, Bavaria) were placed in each participating yard and allowed to run for 24 hours. Traps were each baited using a BG Lure and were placed at the same locations in the yards at the same time each week. Mosquitoes were collected weekly starting one week before the applications, shortly after the application was applied, and on weeks 1, 2, 3, 4, 6, and 8 post-application. The number of mosquitoes was tracked for each trap at each location. Due to the length of the trapping period, some specimens were badly damaged, therefore all analyses were only performed on identifications at the genus level. Approximately 95% of mosquitoes were *Aedes*, ~5% were *Culex*, and <1% were *Toxorhynchites* (data not shown).

Experimental treatments

Properties were assigned either to a treatment group (Demand CS®: 7.9% lambda-cyhalothrin; Syngenta Crop Protection, Greensboro, NC) at 6.25 ml [AI]/liter or a control group using only water. Pre-treatment catches were determined to be statistically similar across all participating properties, and therefore treatment groups were randomly assigned. The spray treatments were applied using a backpack mist blower (SR200, Stihl Corp., Virginia Beach, VA) and by the same pest control operator from the collaborating company on August 6th and 7th (Bug Man Exterminating, T. Nininger, personal communication). No additional applications of any treatments were applied for any of the participating properties for the duration of the study.

Homeowner questionnaire

Participating homeowners were asked to take 3 voluntary surveys consisting of three questions regarding their opinions about insecticide applications used to control mosquitoes. The surveys were conducted pre-treatment, mid-treatment, and post-treatment. These questions are graded on individual Likert scales and are listed under Extra Documents 1.

Statistical analysis

The effects of treatment and trapping period on the total number of mosquitoes caught per location over time were determined using a linear mixed model for repeated measures ANOVA with an autoregressive covariance structure (Littell et al. 2000, Traver et al. 2018). Since trap catches were ~95% *Aedes* mosquitoes (data not shown), total counts were grouped and used for analysis. In the model, treatment (Demand CS or water), trapping period (Monday/Tuesday or Tuesday/Wednesday), and sampling week (-1, 0, 1, 2, 3, 4, 6, and 8), and their 2- and 3-way interactions were the fixed effect factors. In the presence of a significant 3-way interaction, we analyzed one of the 2-way interactions at each level of the third factor and repeated this process for each of the other two factors, as suggested by Ott and Longnecker (2001). Post hoc multiple comparison tests were carried out with Student's t-tests or Tukey's HSD, where appropriate. Ordinal survey data were analyzed with Student's t-test. All statistical analyses were carried out using JMP Pro v14 (SAS, 2019), at a significance level of $\alpha = 0.05$.

Environmental data

Mean temperature, mean percent relative humidity, and total precipitation during the study period were 23.6 °C, 76.6%, and 443 mm, respectively (Table 4.1). Data were retrieved from Weather Underground (Preston Park station: KVAROANO50) (Weather Underground 2019). This weather station is located in the relative center of the treatment area. These temperatures are slightly higher the historical average temperatures for this time period (~22.2°C) and drastically higher than the typical average rainfall in the area (~152 mm), according to climate averages for the Roanoke, VA area (weatherspark.com 2020).

4.4 Results

Effect of treatment

Data were analyzed to determine the effects of treatments (Demand CS at 6.25 ml[AI]/liter or water), trapping period (Monday/Tuesday or Tuesday/Wednesday), and sampling week (-1, 0, 1, 2, 3, 4, and 8) on mosquito abundance as indicated by weekly trapping counts. The analysis showed that there were no significant interactions between any of the above factors ($P > 0.05$). Significant effects were observed only between treatment groups ($F1, 83.0 = 17.455$, $P < 0.0001$; Fig 4.2). A total of 922 female mosquitoes were collected throughout the study, with treatment properties averaging 4.2 mosquitoes per night per property and control properties averaging 17.6 mosquitoes per night per property. Mosquito catch numbers were similar the week before treatments were applied, according to samples taken, which indicates that the residences in this study originally possessed similar local populations of mosquitoes prior to treatment. Population trends represented by trap counts over the sampling period were not significantly different across weeks (Fig 4.3). By the end of the study, there were more mosquitoes present compared to the starting points of the residences, but this difference was not statistically significant.

Participant survey responses

There were no significant differences between the control group participants and the treatment group participants for the pre-treatment survey responses (Fig 4.4, $P> 0.05$), which indicates similar biases for starting conditions.

In the mid-treatment survey, there were significant differences in responses to questions 1 and 3 (Fig 4.5; F= 7.053, P= 0.0160 and F= 2.201, P= 0.0457, respectively). In mid-treatment survey question 1, control properties noticed significantly more mosquitoes at their residences, as compared to the reports from treatment properties (Fig 4.5). Mid-treatment question 3 referred to the satisfaction of the homeowner if they had paid for the treatment they received; treatment properties reported great satisfaction with their treatments, but control properties only reported a neutral or negative assessment (Fig 4.5).

Post-treatment survey responses only differed significantly on question two, which referred to the homeowner's assessment of the mosquito population present in their yard at the end of the study. Control properties reported that they needed repellents or other additional control methods to remain active outside, but treatment properties reported that mosquitoes were not a problem at the residence (Fig 4.6; F= 3.657, P=0.0170).

4.5 Discussion

The use of residual insecticide applications to manage adult mosquito population pressure is a commonly recommended practice for mosquito control companies (Anderson et al. 1991, Cilek and Hallmon 2006, Trout et al. 2007, AMCA 2017). Barrier spray application is one aspect of integrated mosquito control programs, alongside the use of repellents for personal protection, managing mosquito larval habitats in the local area, and treating known larval habitats with larvicides (AMCA 2017). Although barrier sprays applied using a backpack sprayer can be costly and labor-intensive for large-scale applications, there is evidence that supports their use at the residential scale (Qualls et al. 2012, Richards et al. 2017). This experiment was designed to evaluate the residual effectiveness of Demand CS barrier spray treatments when applied to suburban yards in Roanoke, Virginia.

The mean number of mosquitoes captured over the course of the study was significantly different between treatment and control properties (Fig 4.2). Control and treatment mosquito populations were similar one week prior to treatment applications, suggesting similar initial mosquito populations at the properties before involvement in the study (data not shown). The second week after treatment was applied experienced a large rainfall event, which is likely the cause of the reduced catch numbers during that time (Fig 4.3). Lambda-cyhalothrin was selected for this experiment because previous research indicated it would perform effectively as a barrier spray treatment (Li et al. 2010; Unlu et al. 2017; McMillan et al. 2018), and better than previously examined formulations of bifenthrin and deltamethrin (McMillan et al. unpublished data). Over the course of the study, the Demand CS formulation of lambda-cyhalothrin provided a significant reduction in local mosquito populations inside the barrier, compared to the populations observed on the control properties. These results are consistent with previous research regarding the residual effectiveness of lambda-cyhalothrin that showed that the Demand CS formulation was effective against *Ae. albopictus* females for approximately eight weeks under field conditions (McMillan et al. 2018).

Adult female mosquito abundance did not show significant differences between weeks, despite seasonal variation in mosquito populations. This study shows that although some seasonal changes were observed, applications of Demand CS as a barrier treatment significantly reduced the magnitude of the local mosquito populations. Total catch numbers were highest in late August/early September, which was a time period preceded by the largest amount of rainfall event of the study (week 2). This pattern is consistent with previous reported phenology of *Ae. albopictus* (Barker et al. 2003), which made up ~60% of all trap catches in this study (data not shown). Rainfall was unusually high throughout the study, with over a 50% increase in August and nearly a 300% increase in September, as compared to historical rainfall data (weatherspark.com 2020).

Survey responses to the pre-treatment survey were similar for all of the participants: homeowners were not overly concerned about using chemical methods to control mosquitoes (in general or at their residence), and all homeowners identified their location as having a noticeable mosquito population. Homeowners were not aware if they received the Demand CS treatment or the water control, and received their assigned treatment free of charge. Differences in survey responses for the mid-treatment surveys between the two treatment groups were expected; participants in the control groups reported higher mosquito pressure from their residence and a general dissatisfaction with the treatment as compared to homeowners who received the Demand CS barrier spray treatment. Post-treatment survey question two continued this trend for both treatment groups, with control participants reporting a significantly higher abundance of mosquitoes on their property than treatment group reports.

A significant effect from treatment on the populations of mosquitoes captured was observed in this study, and this was reflected by the homeowner survey responses. It should be reiterated that the participants did not know to which group they had been assigned, and yet the treatment groups reported lower mosquito biting pressure than the control group. The results of this experiment provide additional support for the use of a microencapsulated lambda-cyhalothrin formulation as a barrier spray treatment for mosquito control (Trout et al. 2007; McMillan et al. 2018). The microencapsulation technology used in this formulation of lambda-cyhalothrin protects the active ingredient from degradation from the environment (Wege et al. 1999) and is a probable reason for the longevity of the treatment.

In conclusion, Demand CS applied to perimeter foliage/structures at the recommended label rate should reduce the pressure from the local population of adult mosquitoes. As part of an IPM approach, barrier sprays can be used to reduce exposure to the local mosquito population by deterring mosquitoes from resting on treated substrates in the nearby area. This study indicates that such a reduction would persist for at least two months, despite harsh conditions from the environment. Although seasonal variation in the local populations may still occur, they will be significantly reduced compared to residences where no treatment has been applied. This reduces the amount of pesticides required to suppress a population, and could eliminate the need for re-treatments later in the season. These results provide both economic and environmental incentives for pest management companies when evaluating treatment options for residential mosquito control.

Acknowledgements

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4.7 Figures and Tables

Week #	Precipitation (mm)	Temp AVG (°C)	Humidity AVG (RH)
-1	53.8	24.1	96.4
0	23.4	25.5	67.3
1	24.9	24.9	68.0
2	50.3	22.5	70.7
3	20.8	25.5	72.0
4	24.4	24.9	69.9
5	93.5	22.3	87.6
6	114.0	23.5	77.1
7	38.1	19.5	83.9
8	0.3	23.1	73.3
Average:	44.4	24.1	76.6
Total Precipitation:	443.5		

Table 4.1 Weekly rainfall (mm), mean temperature (°C) and relative humidity in Roanoke, VA from 30 July to 3 October, 2018. Data was retrieved from Weather Underground (Preston Park station: KVAROANO50) (Weather Underground 2019).

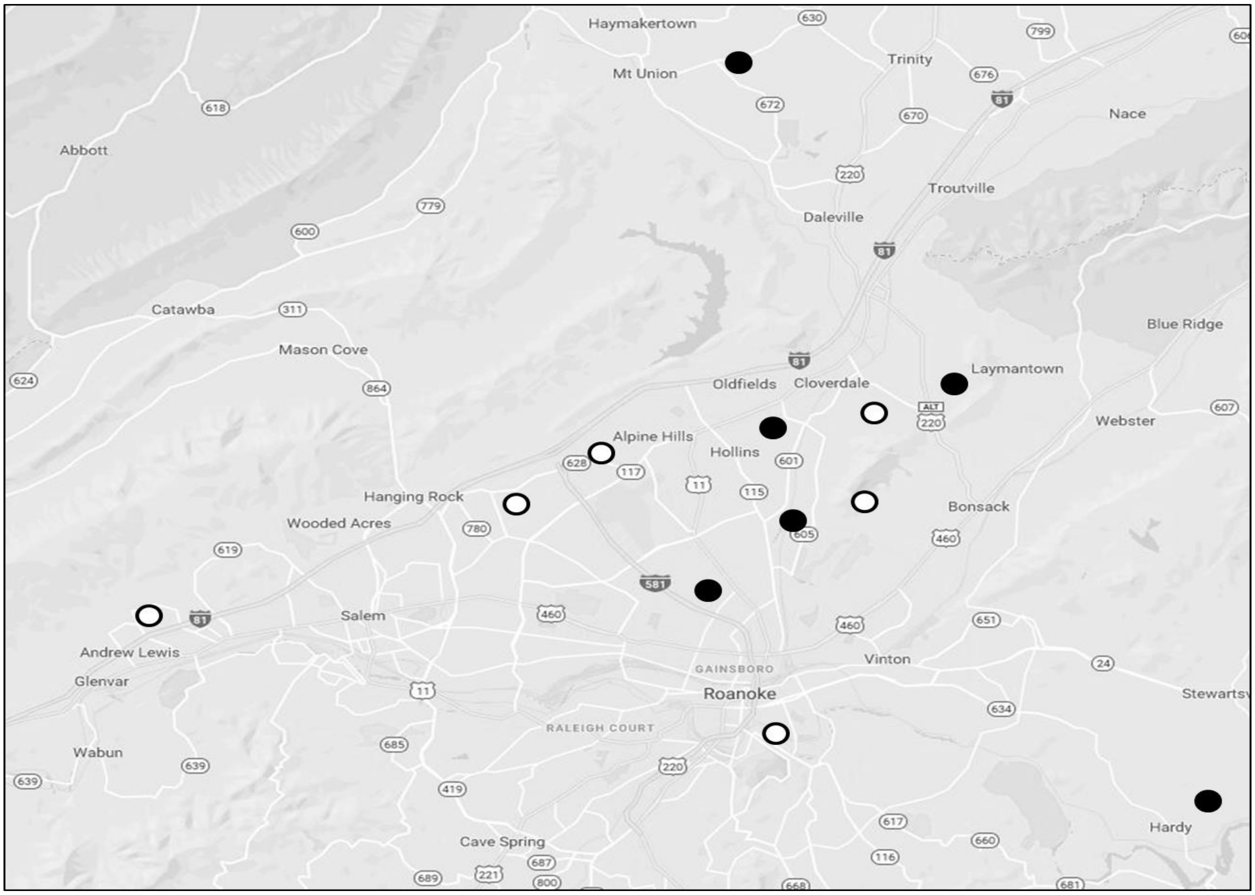


Figure 4.1 Locations of participating properties, all located near the city of Roanoke, Virginia. Dots with a white center represent control properties; dots with a black center represent treatment properties.

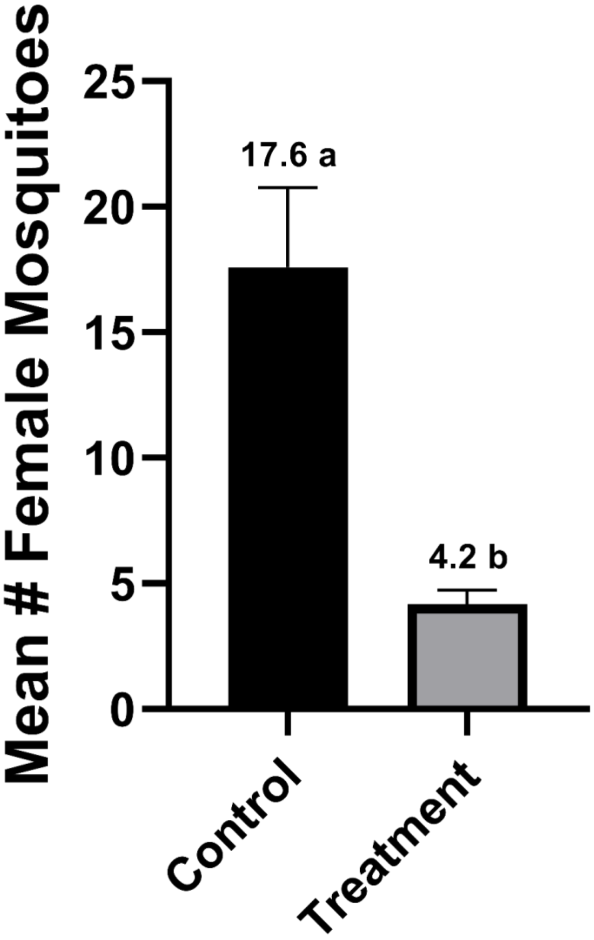


Figure 4.2 Number (mean ± SE) of adult female mosquitoes captured each week per property, sorted by treatment group. Trap catches were averaged across all weeks of the study. Columns followed by different letters are significantly different (Student's t-test; $P<0.05$).

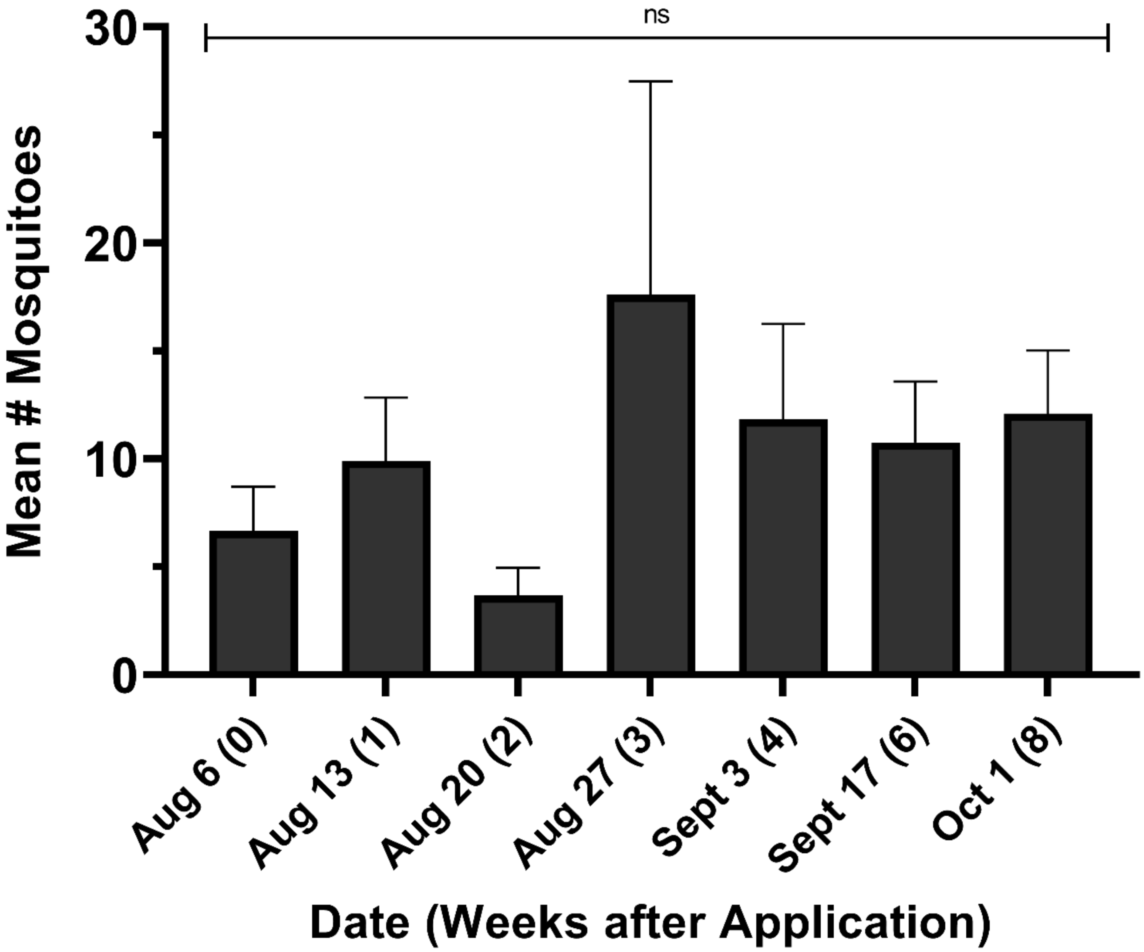


Figure 4.3 Number (mean ± SE) of adult female mosquitoes captured per night per property, sorted by week after treatment application. Weekly trap catches were averaged across all treatment groups. No significant differences were observed for the duration of the study (Tukey's HSD; P>0.05).

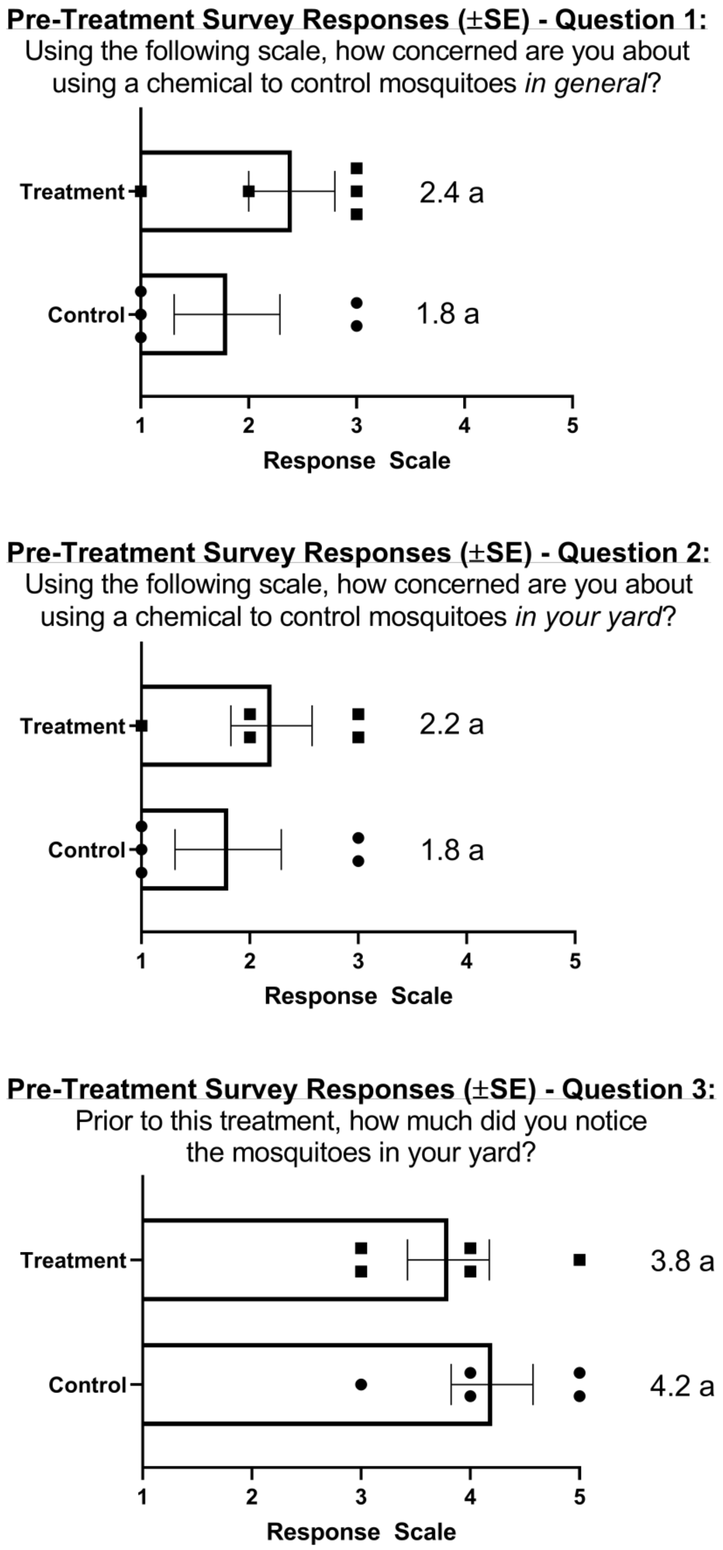
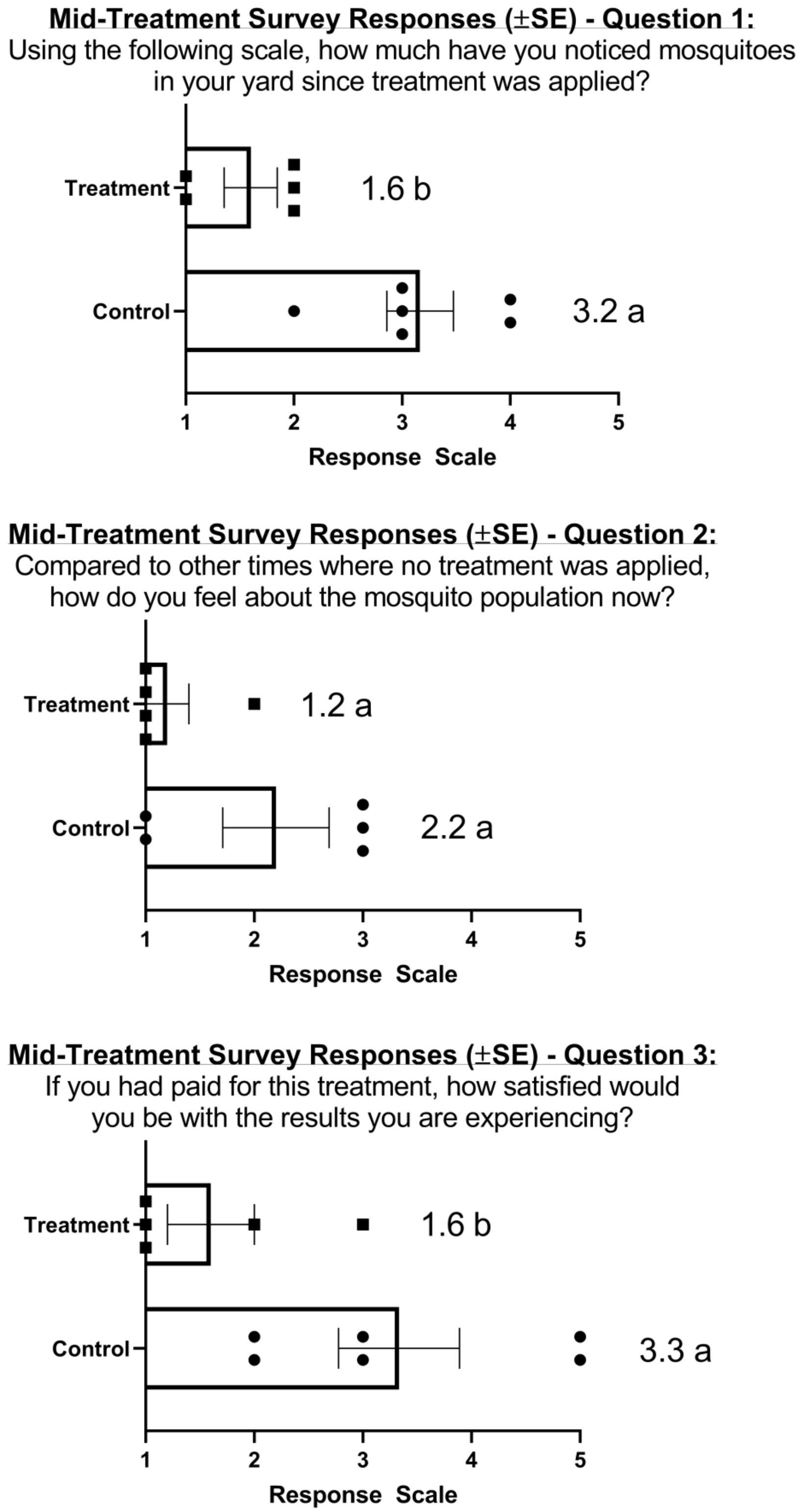


Figure 4.4 Responses to pre-treatment survey questions. Means (± SE) for each graph followed by different letters are significantly different. Student's t-test (P<0.05).


Mid-Treatment Survey Responses (±SE) - Question 1: Using the following scale, how much have you noticed mosquitoes in your yard since treatment was applied?

Mid-Treatment Survey Responses (±SE) - Question 2: Compared to other times where no treatment was applied, how do you feel about the mosquito population now?

Mid-Treatment Survey Responses (±SE) - Question 3: If you had paid for this treatment, how satisfied would you be with the results you are experiencing?

Figure 4.5 Responses to mid-treatment survey questions. Means (± SE) for each graph followed by different letters are significantly different. Student's t-test (P<0.05).

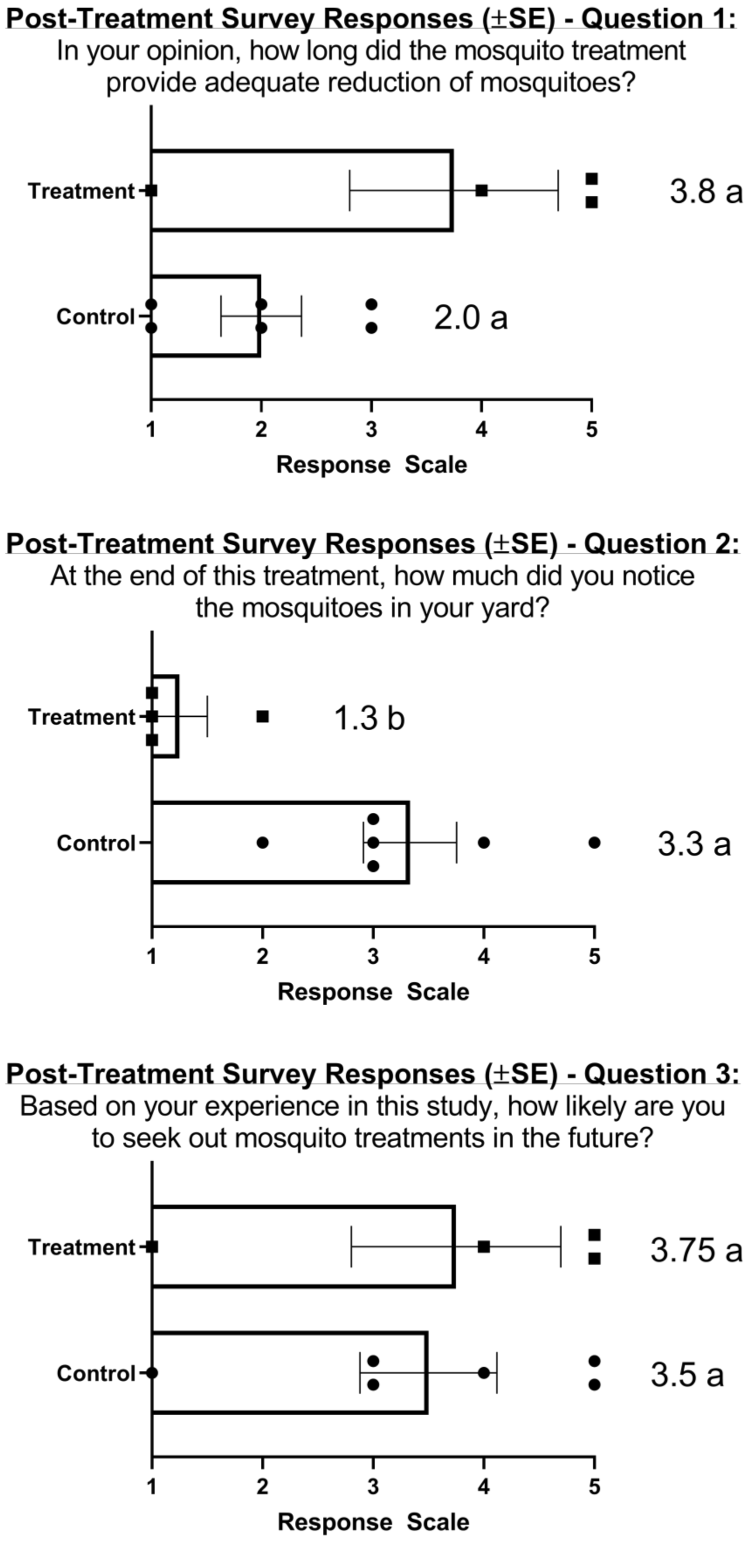


Figure 4.6 Responses to post-treatment survey questions. Means (± SE) for each graph followed by different letters are significantly different. Student's t-test (P<0.05).

4.8 Extra Documents

4.1 *Pre-Treatment Survey:*

1. Using the following scale, how concerned are you about using a chemical to control mosquitoes *in general*?
   1: Not concerned at all.
   2: Concerned a little.
   3: Moderately concerned.
   4: Strongly concerned.
   5: Extremely concerned.
2. Using the following scale, how concerned are you about using a chemical to control mosquitoes *in your yard?*
   1: Not concerned at all.
   2: Concerned a little.
   3: Moderately concerned.
   4: Strongly concerned.
   5: Extremely concerned.
3. Prior to this treatment, how much did you notice the mosquitoes in your yard?
   1: Mosquitoes are not a problem at this property.
   2: There are some, but they usually aren't noticeable.
   3: There are enough that we use repellents or other methods to keep them away.
   4: Mosquitoes are a problem and are difficult to control at this property.
   5: Mosquitoes are so bad that we avoid being outdoors sometimes.

4.2 *Mid-Treatment Survey:*

1. Using the following scale, how much have you noticed mosquitoes in your yard since the treatment was applied?
   1: No mosquitoes present.
   2: Some mosquitoes present.
   3: Mosquitoes are noticeable.
   4: Mosquitoes are noticeable and very abundant.
   5: Mosquito pressure is so great that I do not want to be outside without repellents/protection.
2. Compared to other times where no treatment was applied, how do you feel about the mosquito population *now?*
   1: Mosquitoes are much less present.
   2: Mosquitoes are less present.
   3: Mosquitoes are about the same.
   4: Mosquitoes are slightly worse.
   5: Mosquitoes are much worse.

3. If you had paid for this treatment, how satisfied would you be with the results you are experiencing?
   1: Very satisfied.
   2: Somewhat satisfied.
   3: Neutral.
   4: Somewhat dissatisfied.
   5: Greatly dissatisfied.

4.3 *Post-Treatment Survey:*

1. In your opinion, how long did the mosquito treatment provide adequate reduction of mosquitoes?
   1: Not long at all (0-1 weeks).
   2: Short timeframe (2-3 weeks).
   3: Medium timeframe (4-5 weeks).
   4: Long timeframe (6-7 weeks).
   5: Extremely long timeframe (8+ weeks).
2. At the end of this treatment, how much did you notice the mosquitoes in your yard?
   1: Mosquitoes are not a problem at this property.
   2: There are some, but they usually aren’t noticeable.
   3: There are enough that we use repellents or other methods to keep them away.
   4: Mosquitoes are a problem and are difficult to control at this property.
   5: Mosquitoes are so bad that we avoid being outdoors sometimes.
3. Based on your experience in this study, how likely are you to seek out mosquito treatments in the future?
   1: Not likely.
   2: Somewhat unlikely.
   3: Neutral.
   4: Somewhat likely.
   5: Very likely

Chapter 5
Summary and Conclusions

5.1 Summary and Conclusions

The Asian tiger mosquito, *Aedes albopictus*, is a nuisance and vector mosquito that is a public health concern across the globe. To protect people from potential disease exposure, pest management companies, and mosquito control programs have utilized a variety of different treatment options. An increasingly popular option in the United States is using residual insecticides applied to resting substrates to create a barrier of treatment, and this helps to limit exposure to mosquitoes inside the treated area. Research has been conducted to evaluate the effectiveness of these treatments in the field (Trout et al. 2007, Amoo et al. 2008, Doyle et al. 2009, Richards et al. 2017), but many active ingredients and mosquito species have yet to be examined in this way. Expanding on these studies, we designed experiments to investigate the following subsequent questions:

Are pyrethroid barrier spray applications effective against Aedes albopictus*, and if so, for how long?*

Pyrethroid barrier spray applications are effective against *Ae. albopictus* females, but the length of this effectiveness varies between active ingredients (AI) and environmental factors. We exposed female *Ae. albopictus* mosquitoes to plant foliage treated with residual pyrethroid applications (Demand® CS [AI lambda-cyhalothrin], Talstar® Professional [AI bifenthrin], and Suspend® Polyzone® [AI deltamethrin]) and observed the proportion knockdown after 1 hour and mortality after 24 hours. Two bioassays were performed simultaneously: one group was exposed to the treated substrates for the entire observation time and the other was exposed for only five minutes before being transferred to a sterile environment for observation. As expected, female *Ae. albopictus* that were exposed to a treated substrate for only 5 minutes demonstrated significantly lower knockdown after 1 hour and mortality after 24 hours than those that were exposed for the full 1 hour and 24 hours. It is therefore important that bioassays provide a realistic exposure timeframe, so as to not overstate the expected level of control when applied in the field. In addition, active ingredients and their various formulations should be selected based on the desired timeframe of control. These data suggest that applications of Demand CS (AI lambda-cyhalothrin) should induce significant knockdown and mortality in female *Ae. albopictus* for at least eight weeks after application as a barrier spray treatment. Applications of Talstar Professional (AI bifenthrin) should result in significant knockdown/mortality for around two weeks under those same conditions, and applications of Suspend Polyzone (AI deltamethrin) may not provide significant levels of control in the field, due to low knockdown and mortality measurements observed from the bioassays with limited exposure timeframes. This experiment was conducted as a semi-field trial, and therefore the environmental conditions that were present may not relate to all barrier spray applications of these active ingredients/formulations. Further investigation into the residual efficacies of these and other active ingredients/formulations should be conducted to provide the most complete information regarding treatment effectiveness in the field. Our study contributes to this body of evidence by providing information on female *Ae. albopictus* knockdown and mortality when exposed to Demand CS (AI lambda-cyhalothrin), Talstar Professional (AI bifenthrin), or Suspend Polyzone (AI deltamethrin) when applied as a barrier spray treatment in Southwestern Virginia (Chapter 2).

Does the foliage to which a pyrethroid barrier treatment is applied impact the effectiveness against Aedes albopictus*?*

The bioassays mentioned above were conducted using five common plants for residential landscaping (arborvitae, boxwood, Japanese honeysuckle, rhododendron, and zebra grass), and differences in female *Ae. albopictus* knockdown/mortality were observed after one hour and 24 hours, respectively. It was determined that the species of plant to which treatments were applied had a significant effect on the efficacy of the three pyrethroid treatments (Demand CS [AI lambda-cyhalothrin], Talstar Professional [AI bifenthrin], and Suspend Polyzone [AI deltamethrin]), with rhododendron and zebra grass showing the highest knockdown/mortality proportions and Japanese honeysuckle showing the lowest (Chapter 2). We hypothesize, alongside Doyle et al. (2009), that this observed effect may be due, at least in part, to the treated surface area available to the mosquitoes. As such, additional experimentation was conducted to examine the effect of plant species on residual pyrethroid applications and was performed controlling for available surface area. Bioassays were conducted again using three plants species (dogwood, rhododendron, and American holly) and the same three residual pyrethroid treatments. This experiment determined that plant species still had a significant effect on the examined pyrethroid treatments, but the effect was not uniform across all active ingredients. Demand CS was not significantly impacted by the plant substrate, Talstar Professional varied significantly for the observed mortality, and Suspend Polyzone varied significantly for both observed knockdown and mortality (Chapter 3). These results contribute to the existing body of evidence regarding substrate impact on pyrethroid barrier spray effectiveness, though many more substrate/active ingredient combinations should be examined to better account for variables in the field (Chapters 2 and 3).

Does the blood meal status of an adult female Aedes albopictus *influence its susceptibility to pyrethroid barrier treatments?*

Bioassays were conducted to compare the susceptibility of female *Ae. albopictus* that had recently taken a blood meal to those that had not fed. These data show that the presence of a recently-imbibed blood meal does make female *Ae. albopictus* more susceptible to pyrethroid barrier spray treatments, both in proportion knockdown at one hour and mortality at 24 hours post-exposure. This increase in susceptibility to pyrethroid treatments was not uniform across the three examined pyrethroids (Demand CS [AI lambda-cyhalothrin], Talstar Professional [AI bifenthrin], and Suspend Polyzone [AI deltamethrin]). Demand CS and Suspend Polyzone treatments were statistically similar for both proportion knockdown and proportion mortality against blood-fed *Ae. albopictus* females, but Demand CS caused significantly higher knockdown and mortality against unfed mosquitoes. Barrier spray treatment applications of Talstar Professional resulted in significantly lower proportions of knockdown and mortality than either of the other two examined insecticides, regardless of the presence/absence of a blood meal. There is limited information currently available regarding the relationship between a freshly-acquired blood meal and the efficacy of a residual pyrethroid application. Understanding this relationship has a potential impact on public health recommendations in response to a mosquito-borne disease outbreak, and the data described here contribute to this foundational knowledge (Chapter 3).

Do barrier spray treatments reduce mosquito populations inside the desired area when applied in a suburban setting?

Properties in Roanoke, Virginia were treated with Demand CS [AI lambda-cyhalothrin] and compared to local control properties in peak mosquito season for the area (late summer). Mosquito catch numbers were recorded for eight weeks and compared across treatment groups. It was determined that barrier spray applications of Demand CS provided a significant reduction in mosquito populations inside the treated area for at least eight weeks, and surveys of homeowners who received these treatments corroborate these findings. Throughout the study, both control- and treatment-recipient homeowners were asked to participate in voluntary surveys regarding their impressions of the treatments, but were unaware of the treatment their property received. Homeowner responses supported the results of the trapping; participants that received the Demand CS treatment reported significantly less mosquito pressure at their property, as well as significantly higher satisfaction with the treatment, as compared to the control recipients. These results represent the field efficacy of Demand CS barrier spray treatments, and contribute additional validation for the use of residual pyrethroid treatments applied as barrier sprays. Subsequent research in this area should investigate the field efficacy of other active ingredients/formulations, which would allow mosquito control companies to choose the most effective treatment option on a case-by-case basis for their consumers. Additional environments should also be examined to better understand how the residual efficacy of a treatment changes with environmental factors (Chapter 4).

5.2 References

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