

BIOLOGY AND CONTROL MEASURES FOR *AEDES ALBOPICTUS* (SKUSE), THE
ASIAN TIGER MOSQUITO, IN NORTHEASTERN USA

by

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ABSTRACT OF THE DISSERTATION

Biology and control measures for *Aedes albopictus* (Skuse),

the Asian tiger mosquito, in northeastern USA

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Aedes albopictus is an invasive species with expanding geographic range and involvement in mosquito-borne diseases. Host selection patterns by invasive mosquitoes are important because they increase endemic disease transmission and drive outbreaks of exotic pathogens. *Aedes albopictus* has been characterized as an opportunistic feeder but limited information is available on their feeding patterns in temperate regions. Because of the increasing expansion and abundance of *Ae. albopictus* and the escalating diagnoses of exotic pathogens in travelers returning from endemic areas, I investigated the host feeding patterns of this species in newly invaded areas to elucidate its role in disease ecology and assess the public health threat of an exotic arbovirus outbreak.

In Chapter 1, I report the blood meal results from *Ae. albopictus* in New Jersey. I found that *Ae. albopictus* fed exclusively on mammalian hosts with over 90% of their blood meals derived from humans (58%) and domesticated pets (23% cats, 15% dogs). No avian-derived blood meals were detected. The high mammalian affinity of *Ae. albopictus* suggests that this species will be an efficient vector of mammal- and human-

driven zoonoses like dengue and chikungunya viruses but may have limited exposure to endemic avian zoonoses like West Nile virus.

In Chapters 2 and 3, I investigated the penetration, characteristics, and efficacy of a nighttime adulticide application against diurnal populations of *Ae. albopictus*. Adult control of *Ae. albopictus* is difficult because the species occurs primarily within cryptic habitats of residential backyards where obstacles such as buildings can disrupt spray plumes and penetration. I collected aerosol droplets consistently from all habitats, with no significant differences detected between locations within the same application rate. Mid label rates displayed similar droplet density values as max label rates in urban areas. Dual applications at mid label rate spaced one or two days apart accomplished significantly higher reduction (85%) than single full rate applications (73%). Our results demonstrate that nighttime adulticiding is effective in reducing *Ae. albopictus* abundance and highlight its potential use as part of integrated mosquito management programs and during disease epidemics when reducing human illness is of paramount importance.

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Introduction

Mosquitoes are important global vectors of pathogens and arthropod-borne viruses (arboviruses). Container-inhabiting mosquitoes of the genus *Aedes* are important vectors of arboviruses such as chikungunya (CHIK), dengue (DEN), West Nile (WN), and yellow fever (YF). Multivoltine *Aedes* species utilize container habitats by ovipositing desiccation-resistant eggs that survive drought for extended periods of time. Natural containers utilized by these species include bamboo nodes, plant axils, rock pools, and tree holes; however, artificial containers such as discarded tires also provide suitable habitats which mimic natural oviposition sites. The desiccation-resistant eggs of container *Aedes* have facilitated invasion into new areas, primarily through transportation via the international trade in used tires (Reiter and Sprenger 1987). Increased global travel and trade in used tires are major contributing factors for the dispersal of exotic *Aedes* species of medical importance. Moreover, the ubiquity of used tires and other artificial containers in urban/suburban areas prohibit effective control of these medically important species. Larvae of invasive container *Aedes* are often superior competitors and may be responsible for reduction of native mosquitoes in overlapping ranges (Andreadis et al. 2001, Juliano and Philip Lounibos 2005, Rochlin et al. 2013a). The public health threat from exotic species introduction into new areas is evident, and in many cases, vector suppression is the only means to successfully combat exotic diseases.

Aedes albopictus (Skuse), the Asian tiger mosquito, is among the most invasive of all animal species, and perhaps the most invasive of all mosquitoes. The mosquito is considered as one of the "*100 of the World's Worst Invasive Alien Species*" by the World Conservation Union (Luque et al. 2014). The first establishment of *Ae. albopictus* in the

USA was linked to an introduction into Texas during 1985 via used tires shipped from Japan (Sprenger and Wuithiranyagool 1986). Within the last 20 years, the species has spread to 30 states and continues to expand its range, presumably aided by human activities and scrap tire movement on interstate highways (Enserink 2008). The distribution of the species in North America is primarily concentrated around southeastern USA, with a westward range into Texas, and northward into Illinois and New Jersey (Darsie Jr and Ward 2005). The northern range of *Ae. albopictus* is limited by its inability to survive extreme cold (Nawrocki and Hawley 1987), but the species appears to be more temperate and is slowly expanding its geographical range near its northernmost limits (Farajollahi and Nelder 2009, Rochlin et al. 2013b). Larvae are predominantly peridomestic and thrive in artificial containers, but may also be found in rural areas inhabiting natural containers such as tree holes (Bartlett-Healy et al. 2012, Unlu et al. 2013).

The global expansion of *Ae. albopictus* has also continued extensively from its native tropical range in Southeast Asia and the species is now found on every continent except Antarctica (Benedict et al. 2007, Enserink 2008). The last decade, in particular, has seen a dramatic expansion of *Ae. albopictus* into temperate regions of Europe and North America (Farajollahi and Nelder 2009, Schaffner et al. 2009, Rochlin et al. 2013b). In many parts of its expanding range, this species is implicated as a significant vector of emerging and re-emerging arboviruses such as DEN and CHIK.

Although historically not an important vector of CHIK, *Ae. albopictus* has become the principal driver of recent epidemics in Asia and islands in the Indian Ocean because a mutation in the virus envelope protein enhanced transmission efficiency by this

species (Tsetsarkin et al. 2007, de Lamballerie et al. 2008). Autochthonous transmission of CHIK has also been recorded in temperate regions of Italy and France (Rezza et al. 2007, Grandadam et al. 2011) where invasive *Ae. albopictus* have become abundant (Schaffner et al. 2009). *Aedes albopictus* was also the sole vector in local epidemics of dengue in Hawai'i and other regions (Effler et al. 2005, Lambrechts et al. 2010) and is a competent laboratory vector for at least 22 arboviruses (Gratz 2004). The importance of *Ae. albopictus* may be particularly imminent in the case of CHIK, as the virus is explosively spreading in the Caribbean region of the western hemisphere for the first time and could be potentially introduced into mainland USA in the near future (Enserink 2014). Due to the widespread and increasing distribution of *Ae. albopictus* in temperate regions and the escalating diagnoses of exotic pathogens in travelers returning from endemic or epidemic areas (Beltrame et al. 2007, Gibney et al. 2011), the risk of an outbreak in a new area is no longer hypothetical.

Furthermore, because this species thrives in artificial containers found in close association with human peridomestic environments, the public health significance of *Ae. albopictus* may be much greater than expected. But surprisingly, given the vector potential and medical importance of *Ae. albopictus*, few studies have been conducted to investigate the host feeding patterns of this species in its native and expanding geographic range. This is likely because adult *Ae. albopictus* are a difficult species to collect efficiently in traps, and blood fed specimens are especially rare. From the few studies that have been conducted, the precise host feeding preferences of *Ae. albopictus* seem to vary considerably. The species has been generally reported to feed on a wide range of mammals including humans, but will also feed on avian hosts at various

proportions (Savage et al. 1993, Niebylski et al. 1994, Estrada-Franco and Craig 1995, Richards et al. 2006). It has thus been considered an opportunistic feeder and a classic bridge vector candidate between zoonotic arboviruses and humans. However, caution should be taken in labeling *Ae. albopictus* as an efficient bridge vector because the large variation in the feeding plasticity of this species questions the exact role that it may play as an enzootic or epidemic vector of arboviruses. For example, in its native tropical range, *Ae. albopictus* feeds exclusively on humans in Indonesia (Jumali et al. 1979), whereas in Singapore it feeds on humans, oxen, and dogs (Colless 1959). Additionally, studies conducted in Thailand (Sullivan et al. 1971) have reported that *Ae. albopictus* feed on humans, swine, buffalo, dogs, and chickens, while more recent investigations (Ponlawat and Harrington 2005) report that *Ae. albopictus* feeds only on humans, with a few (<6%) double-host blood meals between humans and swine/cat/dog. In temperate Japan, *Ae. albopictus* primarily feed on mammals, with a high propensity for humans, but also on birds and amphibians/reptiles (Kim et al. 2009, Sawabe et al. 2010).

In temperate locations of the expanding range of *Ae. albopictus*, the host preference of this species is also variable. Studies conducted at a tire dump in Missouri, USA, reported that *Ae. albopictus* will feed on birds (17%) but prefer mammals (64%), with 8.2% of those mammalian feedings obtained from humans (Savage et al. 1993). A follow up study conducted in other tire yards and surrounding vegetation of rural and urban habitats in Missouri, Florida, Indiana, Illinois, and Louisiana, USA, concluded that *Ae. albopictus* showed a strong preference for mammals (>94%), with up to 8% human-derived blood meals, while also detecting avian (1%) and reptilian (5%) blood meals (Niebylski et al. 1994). An additional study in suburban landscapes of North Carolina,

USA, reported that *Ae. albopictus* feeds predominately on mammalian hosts (83%), but also on birds (7%), amphibians (2%), and reptiles (2%) (Richards et al. 2006). In Europe, Italian populations of *Ae. albopictus* rarely feed on birds in urban settings, while 99% of specimens have been reported to feed on mammals, with 90% of those mammalian blood meals being derived from humans (Valerio et al. 2010). The same investigators report that in suburban settings of Italy, 7% of *Ae. albopictus* had fed on avian species, while the vast majority of the blood meals were mammalian-derived (95%), with 43% containing human blood (Valerio et al. 2010). Finally, in urban zones of Spain, *Ae. albopictus* obtained blood meals exclusively from humans (100%).

Although it is apparent that *Ae. albopictus* feeds predominantly on mammals, the degree of mammalophagic or anthropophagic host feeding preferences of this species appear location specific. Because of the rapidly expanding range of *Ae. albopictus*, its abundance in metropolitan centers, and its close association with humans in peridomestic habits, combined with the emergence and resurgence of exotic pathogens for which *Ae. albopictus* is a capable vector, it is clear that assessing its host feeding preferences in newly invaded areas is critical to elucidate disease transmission cycles and develop strategies to reduce the local risk of an exotic arbovirus outbreak.

Due to the absence of a vaccine for CHIK, mosquito control, particularly the reduction of biting populations of the primary vector, is the only effective means of reducing CHIK fever cases during an epidemic. Most federal and state guidelines for protecting the public during outbreaks of mosquito-borne diseases recommend adulticides from aircraft and truck-mounted equipment as the most effective method of reducing transmission risk to humans (CDC 2013). These adulticide interventions are

generally applied as ultra-low volume (ULV) cold aerosol sprays during night-time campaigns when a thermal inversion has occurred to keep the insecticide from dispersing upwards and light winds aid in the spread of the insecticide droplets (Mount 1998). But because prior ULV applications have not been efficacious or long lasting in controlling diurnally active urban mosquitoes, such as *Ae. aegypti* (Perich et al. 1990, Reiter 2007) and *Ae. albopictus* (Reiter et al. 1997), they have been declared ineffective in reducing arbovirus transmission (Gubler 1998). Previous researchers have hypothesized that this lack of control may be a result of resting behavior, allowing gravid or engorged females to remain sequestered during nighttime ULV applications in cryptic habitats that are sheltered from the insecticide plume (Focks et al. 1987, Perich et al. 1990, Reiter et al. 1997, Gubler 1998, Reiter 2007). The ineffectiveness of nighttime ULV applications against diurnal mosquitoes has become the conventional wisdom within the modern vector control community in the USA and many mosquito abatement programs simply do not attempt to adulticide against *Ae. albopictus*.

But new formulations, equipment, and techniques are providing much needed alternatives for efficacious control on container-inhabiting *Aedes*. DUET™ Dual-action Adulticide (Clarke, Roselle, IL, USA) is a newly available adulticide for mosquito control that causes a benign agitation [a non-biting excitation of mosquitoes] potentially flushing mosquitoes from resting places and increasing contact with airborne droplets that are more likely to impinge on flying adults (Cooperband et al. 2010). DUET adulticide combines the pyrethroids sumithrin and prallethrin with the synergist piperonyl butoxide. Prallethrin is reported to induce an excitatory response at sublethal concentrations and may drive mosquitoes from a resting state and expose them to lethal

doses of airborne sumithrin and piperonyl butoxide (Cooperband et al. 2010, Clark et al. 2013). This adulticide may have advantages against not only resting gravid or engorged mosquitoes but also against diurnal mosquitoes such as *Ae. albopictus* which may be inactive during routine nighttime ULV applications by mosquito abatement programs.

But crucial information is lacking regarding penetration and density of aerosolized spray droplets within urban and suburban environments where buildings and vegetation can disrupt the movement of the spray plume. Few studies have been conducted to evaluate aerosolized droplet dynamics and characterization during real world spray applications. Movement of aerosols in urban habitats is even more rare (Perich et al. 1992, Perich et al. 2000). Investigations into the dispersal of adulticides more frequently occur under open field or vegetative canopies, because of the simplicity of these models, and then those theories have been applied to urban habitats (Curtis and Mason 1988, Barber et al. 2007, Bonds 2012). Additionally, some researchers have reported that to achieve the same efficacy in dense vegetation or urban habitats (versus open field habitats), application rates would have to be increased several fold (Rathburn Jr and Dukes 1989, Mount 1998). But there is a conflicting increase in the public awareness and environmental concerns regarding insecticides versus the imminent risk to public health of an *Ae. albopictus*-driven arboviral epidemic. Consequently vector control officials must be prepared in all aspects of their integrated mosquito management (IMM) approaches to intervene with the most efficacious products and application strategies. A critical need exists for novel methods of insecticide application or new formulations to achieve successful control while maintaining environmental stewardship and accountability.

Because *Ae. albopictus* populations have exponentially grown in New Jersey in the last decade, creating a formidable challenge for vector control programs, and because the potential for introduction of an exotic arbovirus such as CHIK is high within the urban landscape of northeastern USA, I undertook my investigations to answer critical questions regarding the biology and potential control of this species. To understand the role of *Ae. albopictus* in endemic and exotic disease ecology and assess the public health threat of an introduced arbovirus outbreak, I investigated the host feeding patterns of this species in the northernmost limit of its geographic range in the USA (Chapter 1). To determine the utility of a truck-mounted cold aerosol ULV adulticide within urban and suburban environments, I investigated the penetration and characteristics of aerosol sprays into cryptic habitats where buildings and vegetation can disrupt spray plumes (Chapter 2). To determine the efficacy of nighttime ULV adulticides in peridomestic environments, I investigated the impact (reduction) against diurnal biting populations of *Ae. albopictus* using two different application rates and methods (Chapter 3).

Chapter 1

Comparative host feeding patterns of *Aedes albopictus* (Skuse) in urban and suburban New Jersey and implications for mosquito-borne disease transmission¹

Abstract

Aedes albopictus is an invasive species which continues expanding its geographic range and involvement in mosquito-borne diseases such as chikungunya and dengue. Host selection patterns by invasive mosquitoes are critically important because they increase endemic disease transmission and drive outbreaks of exotic pathogens. Traditionally, *Ae. albopictus* has been characterized as an opportunistic feeder, primarily feeding on mammalian hosts but occasionally acquiring blood from avian sources as well. However, limited information is available on their feeding patterns in temperate regions of their expanded range. Because of the increasing expansion and abundance of *Ae. albopictus* and the escalating diagnoses of exotic pathogens in travelers returning from endemic areas, we investigated the host feeding patterns of this species in newly invaded areas to elucidate its role in disease ecology and assess the public health threat of an exotic arbovirus outbreak. We identified the vertebrate source of 165 blood meals in *Ae. albopictus* collected between 2008 and 2011 from urban and suburban areas in northeastern USA using a network of Biogents Sentinel traps, which enhance *Ae. albopictus* capture counts. We also analyzed blooded *Culex* mosquitoes collected alongside *Ae. albopictus* in order to examine the degree to which trap type may bias the composition of the community of blood sources. We found no evidence of bias since as

¹Faraji, A., A. Egizi, D. M. Fonseca, I. Unlu, T. Crepeau, S. P. Healy, and R. Gaugler. 2014. Comparative host feeding patterns of the Asian tiger mosquito, *Aedes albopictus*, in urban and suburban northeastern USA and implications for disease transmission. PLoS Neg. Trop. Dis. *In press*.

expected *Culex* blood meals were predominantly from birds (n=149, 93.7%) with only a small proportion feeding on mammals (n=10, 6.3%). In contrast, *Aedes albopictus* fed exclusively on mammalian hosts with over 90% of their blood meals derived from humans (n=96, 58.2%) and domesticated pets (n=38, 23.0% cats; and n=24, 14.6% dogs). *Aedes albopictus* fed from humans significantly more often in suburban than in urban areas (χ^2 , p = 0.004) and cat-derived blood meals were greater in urban habitats (χ^2 , p = 0.022). Avian-derived blood meals were not detected in any of the *Ae. albopictus* tested. The high mammalian affinity of *Ae. albopictus* suggests that this species will be an efficient vector of mammal- and human-driven zoonoses such as La Crosse, dengue, and chikungunya viruses. The lack of blood meals obtained from birds by *Ae. albopictus* suggest that this species may have limited exposure to endemic avian zoonoses such as St. Louis encephalitis and West Nile virus, which already circulate in the USA. However, growing populations of *Ae. albopictus* in major metropolitan urban and suburban centers, make a large autochthonous outbreak of an arbovirus such as chikungunya or dengue viruses a clear and present danger. Given the difficulties of *Ae. albopictus* suppression, we recommend that public health practitioners and policy makers install proactive measures for the imminent mitigation of an exotic pathogen outbreak.

Introduction

Understanding the blood feeding patterns of mosquitoes is of paramount importance in determining their vector status in the maintenance and epidemic transmission of arboviruses. Blood feeding patterns of mosquito vectors provide insight into the ecological transmission cycles of pathogens and lead to more efficient disease

and vector control measures for the benefit of animal and human health. For invasive mosquitoes with expanding geographic ranges, such as *Aedes albopictus* (Skuse), the specific blood-hosts impact endemic diseases and can lead to the epidemic transmission of exotic pathogens.

The Asian tiger mosquito, *Ae. albopictus*, has dispersed extensively from its native tropical range in Southeast Asia and is now found on every continent except Antarctica (Benedict et al. 2007, Enserink 2008). The last decade has seen a dramatic expansion of *Ae. albopictus* into temperate regions of Europe and North America (Farajollahi and Nelder 2009, Schaffner et al. 2009, Rochlin et al. 2013b). In many parts of its expanded range, this species is implicated as a significant vector of emerging and re-emerging arboviruses such as dengue (DENV) and chikungunya (CHIKV).

Although historically not an important vector of CHIKV, *Ae. albopictus* has become the principal driver of recent epidemics in Asia and islands in the Indian Ocean because of a mutation in the virus envelope protein enhanced transmission efficiency by this species (Tsetsarkin et al. 2007, de Lamballerie et al. 2008). Autochthonous transmission of CHIKV has also been recorded in temperate regions of Italy and France (Rezza et al. 2007, Grandadam et al. 2011) where invasive *Ae. albopictus* have become abundant (Schaffner et al. 2009). *Aedes albopictus* was also the sole vector in local epidemics of dengue in Hawai'i and other regions (Effler et al. 2005, Lambrechts et al. 2010) and is a competent laboratory vector for at least 22 arboviruses (Gratz 2004). Due to the widespread and increasing distribution of *Ae. albopictus* in temperate regions and the escalating diagnoses of exotic pathogens in travelers returning from endemic or epidemic areas (Beltrame et al. 2007, Gibney et al. 2011), the risk of an outbreak in a

new area is no longer hypothetical. Furthermore, because this species thrives in artificial containers found in close association with human peridomestic environments, it is essential to fully investigate the host feeding patterns of *Ae. albopictus* in order to completely understand its role in disease ecology and public health significance.

Surprisingly, given the vector potential and medical importance of *Ae. albopictus*, few studies have been conducted to investigate the host feeding patterns of this species in its native and expanding geographic range. This is likely because adult *Ae. albopictus* are a difficult species to collect efficiently in traps, and blood fed specimens are especially rare. From the few studies that have been conducted, the precise host feeding preferences of *Ae. albopictus* seem to vary considerably (Table 1.1). The species has been generally reported to feed on a wide range of mammals including humans, but will also feed on avian hosts at various proportions, and has even been incriminated to feed on amphibians and reptiles (Colless 1959, Hess et al. 1968, Tempelis et al. 1970, Hawley 1988, Savage et al. 1993, Niebylski et al. 1994, Estrada-Franco and Craig 1995, Tandon and Ray 2000, Gomes et al. 2003, Almeida et al. 2005, Gingrich and Williams 2005, Ponlawat and Harrington 2005, Richards et al. 2006, Dennett et al. 2007, Kim et al. 2009, Sawabe et al. 2010, Valerio et al. 2010, Muñoz et al. 2011, Kamgang et al. 2012, Tuten et al. 2012). It has thus been considered an opportunistic feeder and a classic bridge vector candidate between zoonotic arboviruses and humans. However, caution should be taken in labeling *Ae. albopictus* as an efficient bridge vector because the large variation in the feeding plasticity of this species questions the exact role that it may play as an enzootic or epidemic vector of arboviruses. For example, in its native tropical range, *Ae. albopictus* feeds exclusively on humans in Indonesia (Jumali et al. 1979),

whereas in Singapore it feeds on humans, oxen, and dogs (Colless 1959). Additionally, studies conducted in Thailand (Sullivan et al. 1971) have reported that *Ae. albopictus* feed on humans, swine, buffalo, dogs, and chickens, while more recent investigations (Ponlawat and Harrington 2005) report that *Ae. albopictus* feeds only on humans, with a few (<6%) double-host blood meals between humans and swine/cat/dog. In temperate Japan, *Ae. albopictus* primarily feed on mammals, with a high propensity for humans, but also on birds and amphibians/reptiles (Kim et al. 2009, Sawabe et al. 2010) (Table 1.1). Additionally, since the species is primarily diurnal (Hawley 1988, Estrada-Franco and Craig 1995), host availability during the daytime feeding periods should also be considered.

In temperate locations of the expanding range of *Ae. albopictus*, the host preference of this species is also variable. Studies conducted at a tire dump in Missouri, USA, reported that *Ae. albopictus* will feed on birds (17%) but prefer mammals (64%), with 8.2% of those mammalian feedings obtained from humans (Savage et al. 1993). A follow up study conducted in other tire yards and surrounding vegetation of rural and urban habitats in Missouri, Florida, Indiana, Illinois, and Louisiana, USA, concluded that *Ae. albopictus* showed a strong preference for mammals (>94%), with up to 8% human-derived blood meals, while also detecting avian (1%) and reptilian (5%) blood meals (Niebylski et al. 1994). An additional study in suburban landscapes of North Carolina, USA, reported that *Ae. albopictus* feeds predominately on mammalian hosts (83%), but also on birds (7%), amphibians (2%), and reptiles (2%) (Richards et al. 2006). In Europe, Italian populations of *Ae. albopictus* rarely feed on birds in urban settings, while 99% of specimens have been reported to feed on mammals, with 90% of those mammalian blood

meals being derived from humans (Valerio et al. 2010). The same investigators report that in suburban settings of Italy, 7% of *Ae. albopictus* had fed on avian species, while the vast majority of the blood meals were mammalian-derived (95%), with 43% containing human blood (Valerio et al. 2010). Finally, in urban zones of Spain, *Ae. albopictus* obtained blood meals exclusively from humans (100%) (Muñoz et al. 2011) (Table 1.1).

Although it is apparent that *Ae. albopictus* feeds predominantly on mammals, the degree of mammalophagic or anthropophagic host feeding preferences of this species appear location specific. Because of the rapidly expanding range of *Ae. albopictus*, its abundance in metropolitan centers, and its close association with humans in peridomestic habits, combined with the emergence and resurgence of exotic pathogens for which *Ae. albopictus* is a capable vector, it is clear that assessing its host feeding preferences in newly invaded areas is critical to elucidate disease transmission cycles and develop strategies to reduce the local risk of an exotic arbovirus outbreak. However, the collection of *Aedes* (*Stegomyia*) spp., such as *Ae. albopictus*, has been difficult because standard vector surveillance traps are generally placed 1.5 m above the ground, are operated overnight, and utilize light as an attractant (Farajollahi et al. 2009). Since *Ae. albopictus* is diurnal and not attracted to light, host-seeks near the ground surface, and utilizes visual, in addition to olfactory cues for host location (Hawley 1988, Estrada-Franco and Craig 1995, Kawada et al. 2007) these traps are not an effective way to collect this species. Consequently, most blood meal analyses to date were performed on specimens collected from areas where their densities are very high, such as tire yards and tire dumps (Table 1.1). The creation of newly developed vector surveillance traps, such as the

Biogents Sentinel (BGS) trap, have only recently allowed the collection of large number of *Ae. albopictus* specimens from typical urban and suburban areas for detailed life history studies (Kroeckel et al. 2006). These traps simulate convection currents created by human body heat, utilize lures which mimic human odors, are operated during the day, placed at the ground level, and utilize contrasting black and white markings that provide additional visual cues that may be attractive to *Ae. albopictus* (Kroeckel et al. 2006, Kawada et al. 2007, Farajollahi et al. 2009, Unlu and Farajollahi 2012, Crepeau et al. 2013b).

We investigated the host feeding patterns of *Ae. albopictus* in temperate North America, near the northernmost boundary of established populations in the eastern United States (Farajollahi and Nelder 2009, Rochlin et al. 2013b). We used an extensive network of BGS traps, which enhance *Ae. albopictus* capture counts, to conduct a multi-year collection of blooded mosquitoes (2008-2011) in urban and suburban sites as part of a larger area-wide project aimed at managing the Asian tiger mosquito (Unlu et al. 2011, Fonseca et al. 2013). Additionally, we assayed blood meals from *Culex* mosquitoes collected in the same traps, locations, and dates as *Ae. albopictus* to determine the potential effects of this new trap on the diversity of blood meal sources obtained from the two vectors. We discuss the implications of our results on established and expanding populations of *Ae. albopictus* and the imminent outbreaks of exotic diseases such as chikungunya or dengue fevers in North America.

Materials and Methods

Study Area

All collections were conducted within two counties (Mercer and Monmouth) located in central New Jersey, USA. Mercer County (40° 13' N, 74° 44' W) is highly urban, with 364,883 residents (2009b) and a population density of 630.2 inhabitants per square kilometer. Mercer County and the low-income City of Trenton, where the studies were conducted, have a population density of 4,286.5 /km² (USCB 2009a). The City of Trenton contains typical dense inner city housing, often built as adjoining row homes or duplexes (Farajollahi et al. 2012). Monmouth County (40° 44' N, 74° 17' W) is defined as primarily suburban and is located in east-central New Jersey with a population of 630,380 (2009a). The boroughs on the Raritan Bayshore, within Monmouth County, where the studies were conducted, have an average population of 1,907.4 /km² (2009a). The Raritan Bayshore primarily contains middle income coastal suburban homes which are often interspersed with forest and green space remnants (Unlu et al. 2011). Within each county, three predefined ~1,000-parcel sites (a parcel is a combination of a house and its associated yard space), ranging in area from 1 km² (Mercer) to 2 km² (Monmouth) were chosen for our investigations. Although individual parcel sizes within the study sites in Mercer County were smaller ($199.5 \pm 18.3 \text{ m}^2$) than those in Monmouth County ($571.1 \pm 31.2 \text{ m}^2$), the number of residents within Mercer sites (19,494) were larger than within Monmouth sites (12,743). Every site, within each county, was previously selected to contain similar socioeconomic parameters, geography, human population density, and mosquito abundance. For a detailed description about site selection and the parameters of each individual site, please refer to (Unlu et al. 2011, Fonseca et al. 2013).

Mosquito Surveillance

Mosquitoes were sampled on a weekly basis during 2008-2011 using a network of Biogents Sentinel (BGS) traps (Biogents AG, Regensburg, Germany). Specific details of surveillance protocols are outlined elsewhere (Unlu et al. 2011, Unlu and Farajollahi 2012, Crepeau et al. 2013b, Crepeau et al. 2013a, Fonseca et al. 2013); but briefly, trap locations were chosen by overlaying a grid of specific distance intervals. We used a 175-200 m distance between BGS traps for each site in Mercer County and 200-400 m distances in Monmouth County because of the larger site areas and limiting number of traps in inventory. These distances were based on current knowledge of *Ae. albopictus* flight range (Estrada-Franco and Craig 1995) and the available resources within each county. A total of 36 to 51 BGS traps, depending on the year, were deployed weekly in Mercer County, while 55 to 57 traps were deployed in Monmouth County. Each BGS trap was placed in residential backyards (near vegetation or shade) of each parcel selected, and was operated for 24 hours prior to collection. Each week, traps were placed in the same location within the backyards. The BGS trap was used with a solid BG-lure (Biogents AG, Regensburg, Germany) containing ammonia, lactic acid and fatty acids, components known to be attractive to *Ae. albopictus* (Farajollahi et al. 2009). Although the BGS trap was designed to capture host seeking (unfed) *Aedes* (*Stegomyia*) mosquitoes (Kroeckel et al. 2006), the trap also captures other species such as *Culex* mosquitoes (Farajollahi et al. 2009, Unlu et al. 2011) in addition to occasionally collecting female mosquitoes in varying gonotrophic stages (unengorged, blood fed, black blooded, and gravid). An unengorged or unfed mosquito does not contain visible evidence of blood in the abdomen, while a blood fed mosquito displays a distended abdomen with reddish blood clearly visible. A black blooded specimen has digested most of the blood meal and

retains only a small portion of dark red or black blood visible near the ventral anterior of the abdomen, corresponding with Sella stage VI (Detinova 1962). Gravid specimens have completely digested blood meals and contain visible eggs ready for oviposition.

Collections were placed on dry ice immediately and transported to the laboratory for identification and pooling. Species identification, enumeration, and gonotrophic stage determination was conducted under a dissecting microscope using a chill table to maintain a cold chain. Specimens were stored at -80 °C for subsequent blood meal determination.

Blood Meal Identification from *Ae. albopictus*

Abdomens of blooded *Ae. albopictus* were dissected over a chill table and then extracted using a Qiagen DNeasy Blood and Tissue Kit (Qiagen Sciences, Germantown, MD, USA). Specimens with very small blood remnants or those deemed poorly preserved (desiccated), were not utilized for DNA extraction because those samples rarely yield useful data (Egizi et al. 2013). To avoid contamination, forceps were flamed between extractions. To save time and reagents, we used a strategy that allows rapid identification of human-derived blood meals and mixes between human and non-human mammals (Egizi et al. 2013). This technique identifies human-derived blood meals based on the size of the PCR product on a gel without the need for extensive sequencing, thus drastically reducing costs. A mix between human and non-human blood is detected as two bands, and only the non-human band must be excised from the gel and purified with a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA) prior to sequencing (Egizi et al. 2013). Samples that did not amplify with the above assay were also tested with

previously established primers designed for birds (Cicero and Johnson 2001), reptiles/amphibians (Cupp et al. 2004), and an additional primer set for mammals (Ngo and Kramer 2003). Approximately half of the specimens were tested with all bloodmeal identification methods above to legitimize the use of the rapid-assay (Egizi et al. 2013). To test for contamination, negative controls were employed in all reactions. The negative controls consisted of the PCR master mix with sterile water. Except for the short human-only band obtained with the Egizi et al. assay (Egizi et al. 2013), and when the non-human band was excised from the agarose gel (see above), all PCR products were cleaned with Exo-Sap-IT (USB Products, Cleveland, OH, USA), cycle-sequenced with the forward primer of each pair, and run on capillary automated sequencers. Sequences were BLASTed in GenBank (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) to compare with sequences of known species. Only matches of >98% similarity were identified as the source of the blood meal (Kent 2009).

Molecular Identification and Blood Meal Analyses of *Culex* Mosquitoes

A large number of blooded *Culex* mosquitoes, consisting primarily of *Culex pipiens pipiens* L. and *Culex restuans* Theobald, were also collected by the BGS traps. Because of the difficulty in accurate morphological identification of field-collected specimens due to age or damage (Smith and Fonseca 2004, Harrington and Poulson 2008, Farajollahi et al. 2011) these specimens are often pooled as *Culex* spp. After using a molecular assay to identify all *Culex* mosquitoes to species (Crabtree et al. 1995), we tested blood fed *Culex* specimens from both counties collected in the same traps, locations, and dates as *Ae. albopictus*. *Culex p. pipiens* and *Cx. restuans* were the only

Culex species collected in the BGS traps, and were assayed from Mercer County during 2009-2011 and from Monmouth County during 2008 and 2011. Blooded *Culex* specimens were extracted as described above for *Ae. albopictus*, amplified with the BM primer pair (Kocher et al. 1989), then cleaned, sequenced, and identified as above. The BM primer pair targets a wide range of species, including mammals, birds, and reptiles, but it inadvertently amplifies in *Ae. albopictus* (Egizi et al. 2013) and therefore cannot be used to identify blood meals in that species.

Data Analyses

An independent sample *t*-test was used to determine annual significant differences between the mean numbers of blooded *Ae. albopictus* and *Culex* mosquitoes collected in each county. Spatial differences in the proportion of *Ae. albopictus* feeding on selected host species between the counties was compared by using Pearsons χ^2 analysis for trend. All analyses were performed using IBM SPSS Statistics 21 (IBM, Armonk, NY, USA). Confidence intervals surrounding the estimated proportion of blood meals taken from a given species were calculated using the formula $95\% \text{ CI} = \pm 1.96 \times (\text{square root } p(1-p)/n)$, where p = the proportion of blood meals from a given source, and n = the total number of blood meals identified (Apperson et al. 2004).

Results

Mosquito Surveillance

Our BGS trap surveillance during the active mosquito seasons of 2008-2011 collected 73,828 *Ae. albopictus* females in Mercer and Monmouth Counties (Table 1.2).

A total of 33,392 *Ae. albopictus* were collected in Mercer County, 187 (0.56%) of which were visually determined to contain blood (blood fed or black blooded, hereafter “blooded”); while 40,436 *Ae. albopictus* were collected in Monmouth County, with 219 (0.54%) containing blood. In Mercer County, blooded *Ae. albopictus* were collected during May (n=1, 0.54%), June (13, 6.95%), July (23, 12.30%), August (70, 37.43%), September (61, 32.62%), and October (19, 10.16%). Blooded *Ae. albopictus* in Monmouth County were collected during May (n=4, 1.83%), June (25, 11.42%), July (65, 29.68%), August (72, 32.88%), September (37, 16.90%), and October (16 (7.31%). We also captured 14,989 *Culex* mosquitoes (*Cx. p. pipiens*, *Cx. restuans*, and *Cx. spp.*) from both counties (Table 1.3). The BGS trap is highly specific for capturing host seeking *Ae. albopictus* females, as apparent by the nearly 74,000 specimens of this species that were captured versus the 15,000 specimens of *Culex* mosquitoes (Tables 1.2, 1.3). Interestingly, BGS traps were also efficient at capturing blooded *Ae. albopictus* and *Culex* mosquitoes. No significant differences were observed in the mean number of blooded *Ae. albopictus* versus *Culex* mosquitoes collected in Mercer County during 2008-2009 or 2011, but significantly more blooded *Culex* were collected than *Ae. albopictus* during the 2010 season ($t = 2.258$; $df = 42$; $p = 0.033$). Comparisons in Monmouth County showed no differences between the mean numbers of blooded *Ae. albopictus* and *Culex* mosquitoes collected during 2008, but significantly more blooded *Culex* mosquitoes were collected during 2009 ($t = 3.093$; $df = 46$; $p = 0.005$), 2010 ($t = 3.416$; $df = 48$; $p = 0.002$), and 2011 ($t = 2.137$; $df = 48$; $p = 0.040$).

Blood Meal Identification from *Ae. albopictus*

Of the 406 blooded *Ae. albopictus* collected, 117 individuals were too desiccated and therefore only 289 specimens were suitable for dissection. Subsequently, the blood meal origin of 165 (57.10%) specimens was successfully determined (Tables 1.2, 1.4). In Mercer County, 125 were tested for host blood meal origination with a successful identification from 86 (68.80%) specimens (Table 1.4). In Monmouth County, 164 *Ae. albopictus* were tested, with a successful host determination from 79 (48.17%) of those specimens (Table 1.4).

Aedes albopictus fed exclusively on mammalian hosts in Mercer and Monmouth Counties, with over 84% of all identified blood meals stemming from humans (52.12%), cats (20.61%), or dogs (11.52%) (Table 1.4). Blood meals were also detected from opossums (4.24%), gray squirrels (3.64%), cottontail rabbits (1.21%), and a white-footed mouse (0.61%). A small percentage (6.06%) of double blood meals (from two different host species) were detected in *Ae. albopictus* (4.65% of total in Mercer and 7.60% of total in Monmouth), and all included human blood (human+dog, n=5; human+cat, n=4; human+deer, n=1). The number of *Ae. albopictus* feeding on humans was significantly higher in suburban Monmouth (62%) than in urban Mercer (43%) County locations ($\chi^2 = 8.151$; df = 1; p = 0.004), but significantly more *Ae. albopictus* fed on cats in Mercer than in Monmouth County ($\chi^2 = 5.256$; df = 1; p = 0.022). No significant difference was observed in the number of *Ae. albopictus* feeding on dogs between the two counties. No avian-derived blood meals were detected in any of the *Ae. albopictus* specimens tested.

Human- and cat-derived blood meals in *Ae. albopictus* were detected every month of our studies, while dog-derived blood meals were absent during May (Figure 1.1). Only 2.08% of all human-derived blood meals were detected in May, while the vast majority

was detected during the month of August (38.54%). Four contiguous months (July, August, September, and October) accounted for over 87% of all blood meal collections (Figure 1.1).

Blood Meal Analyses and Molecular Identification of *Culex* Mosquitoes

We collected 745 blooded *Culex* (349 *Cx. p. pipiens*, 181 *Cx. restuans*, 215 *Cx. spp.*) mosquitoes during 2008-2011, and tested a subsample of 198 individuals identified as *Cx. p. pipiens* or *Cx. restuans* for blood meal source determination (Table 1.5). We selected 198 specimens to approximate the number of blood meals identified from *Ae. albopictus* and chose specimens from the same dates and traps as feasible. We were able to identify the blood meal source of 159 (80.30%) samples. Blooded *Cx. p. pipiens* were collected during April (n=1, 0.79%), May (19, 15.08%), June (37, 29.37%), July (26 (20.63%), August (19, 15.08%), September (21, 16.67%), and October (3, 2.38%). Blooded *Cx. restuans* were collected during May (n=10, 30.30%), June (12, 36.36%), July (6, 18.18%), August (2, 6.06%), September (2, 6.06%), and October (1, 3.03%). In Mercer County, specimens were tested from 2009-2011 and resulted in successful host determination from 61 *Cx. p. pipiens* (n=74, 82.43%) and 7 *Cx. restuans* (n=7, 100%). In Monmouth County, the blood meal hosts of 65 *Cx. p. pipiens* (n=80, 81.25%) and 26 *Cx. restuans* (n=37, 70.27%) were determined from 2008 and 2011 (Table 1.5).

Culex mosquitoes were predominately ornithophagic (n=149, 93.71%) with only a small proportion feeding on mammalian hosts (n=10, 6.29%) (Table 1.5). In Mercer County, the avian blood meal hosts of *Cx. p. pipiens* included 16 avian species (88.52%), while mammalian blood meals were obtained from only three species (11.48%).

Mammalian blood was not detected in *Cx. restuans* from Mercer County, whereas avian blood meals were derived from four species (Table 1.5). In Monmouth County, avian hosts of *Cx. p. pipiens* included 12 species (95.39%), while mammalian blood meals were obtained from only two species (4.62%). No mammalian blood was detected in *Cx. restuans* from Monmouth County and avian-derived blood meals were obtained from ten species (Table 1.5).

Discussion

Our investigations provide insight into the host associations of *Ae. albopictus* in the northernmost boundary of their established populations in eastern USA. Currently, about one-third of the human population of 55 million in this region reside in urban areas where *Ae. albopictus* is pervasive. This number is predicted to double under forthcoming climate change scenarios, encompassing all major urban centers and placing over 30 million people under the threat of dense *Ae. albopictus* infestations and potential public health threats from associated emerging mosquito-borne diseases (Rochlin et al. 2013b). Our analyses on the blood feeding behavior of *Ae. albopictus* demonstrate that this species is primarily mammalophilic in peridomestic environments of northeastern USA, and in some locations over 60% of their blood meals are derived from humans.

Host preference studies involving *Ae. albopictus* are often limited by the low sample numbers of blooded mosquitoes that are collected. This is because blooded *Ae. albopictus* have been difficult to collect (Ponlawat and Harrington 2005, Muñoz et al. 2011). Previous sampling methods have often used combinations of aspirators, sweep nets, human baits, sticky traps, carbon dioxide-baited traps, and gravid traps in order to

increase catch counts and as mentioned, often sampled exclusively in high density areas such as tire yards and dumps (Tempelis et al. 1970, Savage et al. 1993, Niebylski et al. 1994, Sawabe et al. 2010, Valerio et al. 2010). But trapping methods may bias results significantly (Thiemann and Reisen 2012), and *Ae. albopictus* is not readily attracted to traditional types of vector surveillance traps (Ponlawat and Harrington 2005, Farajollahi et al. 2009). A consistent sampling tool was not available for *Ae. albopictus* until the development of the BGS trap, which allowed us to sample populations of this species across a large geographic area over multiple years (Unlu et al. 2011, Fonseca et al. 2013). However, unlike blooded or black blooded *Culex* mosquitoes which are easy to discern visually, blooded *Ae. albopictus* (unless fully engorged on fresh blood) are problematic to ascertain. This is because *Ae. albopictus* is a smaller species that imbibes smaller blood meals (Hawley 1988, Estrada-Franco and Craig 1995) or on multiple hosts (Delatte et al. 2010, Farjana and Tuno 2013), and contains a darker integument which hinders accurate detection of blood meals (Muñoz et al. 2011), particularly those in later Sella stages of development (Ponlawat and Harrington 2005). For example, parity studies conducted within our sampling sites on 166 *Ae. albopictus* visually determined as unengorged, detected blood meals or eggs in over 28% of those samples (Farajollahi et al. unpublished data). Our field investigations collected over 400 blooded *Ae. albopictus* during 2008-2011, 289 of which contained amplifiable blood for host determination analyses, with a successful amplification rate of close to 60%. In contrast, amplification rates were much higher for *Culex* mosquitoes (80%), likely because bird blood is nucleated and amplification of target DNA is easier for identification (Kent 2009). Interestingly, we collected twice as many blooded *Culex* mosquitoes than blooded *Ae. albopictus*, despite

the demonstrable specificity of the BGS trap for the latter species. Amplification rates for *Ae. albopictus* also varied between the seasons and counties, as several abnormal weather patterns were experienced, threatening specimen handling and maintenance of the cold chain. The summers of 2010-2011 were particularly detrimental for blooded *Ae. albopictus* because the excessive heat (warmest and 3rd warmest summers on record) may have desiccated specimens much faster in the BGS traps and reduced amplifiable DNA through degradation (http://climate.rutgers.edu/stateclim_v1/data). Nonetheless, successful blood meal results from 165 *Ae. albopictus* across a consistent spatial/temporal span provides valuable insight into the host associations of this species in the northeastern USA.

Our investigations are consistent with previous studies that have shown a high mammalian affinity by invasive *Ae. albopictus* in temperate areas of USA and Europe (Savage et al. 1993, Niebylski et al. 1994, Gingrich and Williams 2005, Richards et al. 2006, Valerio et al. 2010, Muñoz et al. 2011). However, unlike most of these studies, we did not document avian-derived blood meals in any of our *Ae. albopictus* samples despite extensive testing with avian-specific primers. Our findings cannot be attributed to the method of collection, blood meal identification methodology, host availability, or spatial/temporal factors, since the *Culex* mosquitoes collected in the same traps at the same time, were found to feed predominately on birds within our study sites as expected (Apperson et al. 2004, Molaei et al. 2006, Molaei et al. 2008). The lack of blood meals obtained from birds by *Ae. albopictus* suggest that this species may have limited exposure to endemic avian arboviruses, such as West Nile virus (WNV), which is supported by the lack of WNV isolations in over 34,500 specimens assayed in a

complementary study (Armstrong et al. 2013). However, the high mammalian affinity of *Ae. albopictus* suggests that this species may be an efficient vector of mammal-driven zoonoses such as La Crosse virus, and human-driven anthroponoses such as DENV and CHIKV.

Another concern regarding the vectorial capacity of *Ae. albopictus* stems from detection of multiple blood meals from field populations. Previous studies have documented vertebrate blood from more than one host in *Ae. albopictus* throughout its endemic and invasive range (Table 1.1). Our studies detected double blood meals in 6% of the field-collected *Ae. albopictus* specimens, consistent with the 6% to 10% double blood meal proportion rates reported by others (Tandon and Ray 2000, Ponlawat and Harrington 2005, Richards et al. 2006, Sawabe et al. 2010, Valerio et al. 2010). The capacity for *Ae. albopictus* to acquire multiple blood meals, particularly from human and other host species, increases the vector potential of this mosquito because of greater exposure to infected hosts during multiple feedings.

Large proportions of human-derived blood meals have been documented previously in *Ae. albopictus* and a few studies have reported that field populations feed exclusively on humans (Table 1.1), but the use of aspirators and human bait may bias these estimates. Additionally, recent investigations in temperate Italy have shown that *Ae. albopictus* feeding patterns differ between urban and rural habitats, with 90% of blood meals in urban areas from humans and only 20% being human-derived in rural habitats (Valerio et al. 2010). Our results report a significantly higher proportion of human blood meals in *Ae. albopictus* from suburban areas, rather than the densely populated urban areas. This was surprising, because of the higher (>2 times) human population density in

urban Mercer County. However, suburban dwellers often spend more time outdoors gardening or undertaking leisure activities in backyards during daylight hours which will increase exposure. In addition, proportions of *Ae. albopictus* feeding on cats and dogs was higher in urban than suburban sites, likely reflecting large populations of feral cats in urban low income areas (Gehrt et al. 2013) and the fact that often dogs are kept in outside cages or yards for homeowner protection (Unlu and Farajollahi 2012). In contrast, suburban residents primarily keep their pets indoors and availability of these hosts for *Ae. albopictus* may be reduced. The significantly greater anthropophagic behavior of *Ae. albopictus* in more affluent suburban versus low-income urban habitats of northeastern USA indicates that a larger public health concern may exist within suburban landscapes, despite lower human population densities. Higher proportions of *Ae. albopictus* feeding on cats and dogs within urban environs may help fuel local mosquito populations but it may also afford zooprophyllaxis protection for humans during epidemic outbreaks of anthroponoses such as DENV or CHIKV, because it will divert vector feeding to non-susceptible dead-end hosts.

Summary and Public Health Implications

Recent decades have witnessed a dramatic global expansion of *Ae. albopictus* into temperate areas and an increase in locally acquired autochthonous cases of tropical diseases such as DENV and CHIKV (Rezza et al. 2007, Gould et al. 2010, Lambrechts et al. 2010). Because of the increasing abundance of *Ae. albopictus* and the escalating diagnoses of exotic pathogens in travelers returning from endemic or epidemic areas (Gibney et al. 2011), the risk of a tropical disease outbreak in a new area is no longer

speculative. We have shown that in urban and suburban areas of temperate northeastern USA, invasive populations of *Ae. albopictus* fed exclusively on mammalian hosts and that a large proportion (50-60%) fed on human hosts. Although we did not detect any avian-derived blood meals from *Ae. albopictus* during our investigations, the species has been traditionally classified as an opportunistic feeder whose host preference is greatly dependent on the abundance of available local hosts (Hawley 1988, Estrada-Franco and Craig 1995). Our studies indicate that *Ae. albopictus* may play a greater role in anthroponoses disease cycles, such as DENV and CHIKV, and a lesser role in zoonoses involving an avian animal reservoir. However, we cannot rule out the possibility that *Ae. albopictus* may occasionally act as a bridge vector for endemic pathogens such as St. Louis encephalitis virus and WNV by feeding on infected hosts when their abundance is great. Nonetheless, the large and growing populations of *Ae. albopictus* in major metropolitan urban and suburban centers, make a large autochthonous outbreak of an arbovirus such as CHIKV or DENV a clear and present danger. Given the difficulty in successful suppression of *Ae. albopictus* in areas where it has become firmly established (Fonseca et al. 2013, Rochlin et al. 2013b), we strongly recommend further ecological investigations on this species and caution public health practitioners and policy makers to install proactive measures for the imminent mitigation of an exotic pathogen outbreak.

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Table 1.1. Literature review of the host feeding preferences of *Aedes albopictus* in its native and invasive geographic range. All collections were conducted under field settings. Table excludes laboratory or field host-choice experiments.

Geographic Range	Location	Habitat Type	Trap Type	Bloodmeal Assay	No. Identified	Host Class %				Reference
						Mammal (Human)	Avian	Herpitle	>1 Host*	
Native	Japan	Suburban/Urban	CDCLT, SN	PCR	114	84.2 (68.5)	6.1	3.5	6.1	Sawabe <i>et al.</i> 2010
Native	Japan	Rural	BGS, CDCLT, SN	PCR	13	100 (30.8)				Kim <i>et al.</i> 2009
Native	Thailand	Rural	ASP	ELISA	105	100 (94.3)			5.7	Ponkawit & Harrington 2005
Native	China	Rural	ASP	ELISA	48	75.0 (63.9)	25.0			Almeida <i>et al.</i> 2005
Native	India	Cattle shed Suburban Urban	ASP	Precipitin	40	100 (ND)				Tandon & Ray 2000
					162	100 (100)				
					362	81.8 (98.7)	10.5		7.7	
Native	Singapore	Rural	ASP, UTN	Precipitin	37	100 (91.9)				Colless 1959
Old World Invasive	Spain	Urban	BGS	PCR	30	100 (100)				Munoz <i>et al.</i> 2011
Old World Invasive	Cameroon	Rural	SN	ELISA	170	96.3 (100)		0.6	3.1	Kamgang <i>et al.</i> 2012
Old World Invasive	Italy	Rural Urban	ST	ELISA	60	65.0 (30.8)	5.0		30.0	Valerio <i>et al.</i> 2010
					243	92.2 (91.1)	0.8		7.0	
New World Invasive	USA	Zoo	ASP, GT	PCR	5	40.0 (ND)	60.0			Tuten <i>et al.</i> 2012
New World Invasive	USA	Urban	GT	PCR	9	100 (44.4)				Dennett <i>et al.</i> 2007
New World Invasive	USA	Suburban	ASP	ELISA, PCR	1,094	83.1 (24.1)	7.5	3.4	5.1	Richards <i>et al.</i> 2006
New World Invasive	USA	Rural/Suburban	CDCLT, HL, GT	ELISA	22	81.8 (ND)	4.6		13.6	Gingrich & Williams 2005
New World Invasive	Brazil	Urban	ASP, SN	Precipitin	177	97.7 (68.2)	2.3			Gomes <i>et al.</i> 2003
New World Invasive	USA	Rural Urban	ASP	ELISA	93	93.6 (8.1)	1.1	5.4		Niebylski <i>et al.</i> 1994
					152	98.7 (2.0)	1.3			
New World Invasive	USA	Tire dump	ASP, CDCLT, HL, SN	ELISA	139	79.1 (8.2)	20.9			Savage <i>et al.</i> 1993
New World Invasive	USA	Rural	ASP	Precipitin	1,075	93.7 (19.4)	5.8		0.6	Tempelis <i>et al.</i> 1970
New World Invasive	USA	Rural Suburban	ASP	Precipitin	41	27.0 (ND)	73.0			Hess <i>et al.</i> 1968
					14	93.0 (7.1)	7.0			

*Includes specimens with mixed blood meals from more than one vertebrate hosts

ELISA = enzyme-linked immunosorbent assay; ND = non detected; PCR = polymerase chain reaction

ASP = aspirator; BGS = BioGents Sentinel trap; CDCLT = Centers for Disease Control light trap; GT = gravid trap; HL = human landing; SN = sweep net; ST = sticky trap; UTN = unbaited trap net

Table 1.1. Literature review of the host feeding preferences of *Aedes albopictus* in its native and invasive geographic range. All collections were conducted under field settings. Table excludes laboratory or field host-choice experiments.

Geographic Range	Location	Habitat Type	Trap Type	Bloodmeal Assay	No. Identified	Host Class %				Reference
						Mammal (Human)	Avian	Herpitle	>1 Host*	
Native	Japan	Suburban/Urban	CDCLT, SN	PCR	114	84.2 (68.5)	6.1	3.5	6.1	Sawabe <i>et al.</i> 2010
Native	Japan	Rural	BGS, CDCLT, SN	PCR	13	100 (30.8)				Kim <i>et al.</i> 2009
Native	Thailand	Rural	ASP	ELISA	105	100 (94.3)			5.7	Ponkawit & Harrington 2005
Native	China	Rural	ASP	ELISA	48	75.0 (63.9)	25.0			Almeida <i>et al.</i> 2005
Native	India	Cattle shed Suburban Urban	ASP	Precipitin	40	100 (ND)				Tandon & Ray 2000
					162	100 (100)				
					362	81.8 (98.7)	10.5		7.7	
Native	Singapore	Rural	ASP, UTN	Precipitin	37	100 (91.9)				Colless 1959
Old World Invasive	Spain	Urban	BGS	PCR	30	100 (100)				Munoz <i>et al.</i> 2011
Old World Invasive	Cameroon	Rural	SN	ELISA	170	96.3 (100)		0.6	3.1	Kamgang <i>et al.</i> 2012
Old World Invasive	Italy	Rural Urban	ST	ELISA	60	65.0 (30.8)	5.0		30.0	Valerio <i>et al.</i> 2010
					243	92.2 (91.1)	0.8		7.0	
New World Invasive	USA	Zoo	ASP, GT	PCR	5	40.0 (ND)	60.0			Tuten <i>et al.</i> 2012
New World Invasive	USA	Urban	GT	PCR	9	100 (44.4)				Dennett <i>et al.</i> 2007
New World Invasive	USA	Suburban	ASP	ELISA, PCR	1,094	83.1 (24.1)	7.5	3.4	5.1	Richards <i>et al.</i> 2006
New World Invasive	USA	Rural/Suburban	CDCLT, HL, GT	ELISA	22	81.8 (ND)	4.6		13.6	Gingrich & Williams 2005
New World Invasive	Brazil	Urban	ASP, SN	Precipitin	177	97.7 (68.2)	2.3			Gomes <i>et al.</i> 2003
New World Invasive	USA	Rural Urban	ASP	ELISA	93	93.6 (8.1)	1.1	5.4		Niebylski <i>et al.</i> 1994
					152	98.7 (2.0)	1.3			
New World Invasive	USA	Tire dump	ASP, CDCLT, HL, SN	ELISA	139	79.1 (8.2)	20.9			Savage <i>et al.</i> 1993
New World Invasive	USA	Rural	ASP	Precipitin	1,075	93.7 (19.4)	5.8		0.6	Tempelis <i>et al.</i> 1970
New World Invasive	USA	Rural Suburban	ASP	Precipitin	41	27.0 (ND)	73.0			Hess <i>et al.</i> 1968
					14	93.0 (7.1)	7.0			

*Includes specimens with mixed blood meals from more than one vertebrate hosts

ELISA = enzyme-linked immunosorbent assay; ND = non detected; PCR = polymerase chain reaction

ASP = aspirator; BGS = BioGents Sentinel trap; CDCLT = Centers for Disease Control light trap; GT = gravid trap; HL = human landing; SN = sweep net; ST = sticky trap; UTN = unbaited trap net

Table 1.2. Number of *Aedes albopictus* collected by BGS traps in Mercer and Monmouth Counties during 2008-2011.

		<i>Ae. albopictus</i>				
		Unengorged	Blood fed	Black blooded	Gravid	Yearly total
Mercer County	2008	7,862	16	35	8	7,921
	2009	4,716	49	7	7	4,779
	2010	4,698	21	15	55	4,789
	2011	7,887	3	41	7,972	15,903
	Subtotal (%)	25,163 (75.4)	89 (0.3)	98 (0.3)	8,042 (24.1)	33,392
Monmouth County	2008	12,929	98	0	39	13,066
	2009	4,046	14	11	71	4,142
	2010	7,500	17	24	146	7,687
	2011	14,909	22	33	577	15,541
	Subtotal (%)	39,384 (97.4)	151 (0.4)	68 (0.2)	833 (2.1)	40,436
Grand Total (%)		64,547 (87.4)	240 (0.3)	166 (0.2)	8,875 (12.0)	73,828

Table 1.3. Number of *Culex pipiens pipiens* and *Culex restuans* mosquitoes collected by BGS traps in Mercer and Monmouth Counties during 2008-2011. Some specimens were not morphologically identified to species and were enumerated as *Culex* spp. U=unengorged, BF=blood fed, BB=black blooded, G=gravid.

	Cx. p. pipiens					Cx. restuans					Culex spp.					
	U	BF	BB	G	Subtotal	U	BF	BB	G	Subtotal	U	BF	BB	G	Subtotal	
Mercer County	2008	183	1	33	181	398	29	0	0	5	34	287	1	8	22	318
	2009	475	36	39	344	894	67	7	9	91	174	25	1	0	40	66
	2010	538	57	29	272	896	106	6	4	9	125	43	3	1	8	55
	2011	584	0	47	585	1,216	195	0	2	42	239	57	0	2	43	102
	Subtotal (%)	1,780 (52.3)	94 (2.8)	148 (4.4)	1,382 (40.6)	3,404	397 (69.4)	13 (2.3)	15 (2.6)	147 (25.7)	572	412 (76.2)	5 (0.9)	11 (2.0)	113 (20.9)	541
Monmouth County	2008	107	25	6	47	185	81	8	4	39	132	629	27	11	108	775
	2009	266	24	17	82	389	126	13	20	194	353	1,385	17	40	520	1,962
	2010	301	16	6	299	622	451	25	36	469	981	312	23	26	428	789
	2011	358	9	4	535	906	456	21	26	545	1,048	1,273	20	35	1002	2,330
	Subtotal (%)	1,032 (49.1)	74 (3.5)	33 (1.6)	963 (45.8)	2,102	1,114 (44.3)	67 (2.7)	86 (3.4)	1,247 (49.6)	2,514	3,599 (61.5)	87 (1.5)	112 (1.9)	2,058 (35.1)	5,856
Grand Total (%)	2,812 (51.1)	168 (3.1)	181 (3.3)	2,345 (42.6)	5,506	1,511 (49.0)	80 (2.6)	101 (3.3)	1,394 (45.2)	3,086	4,011 (62.7)	92 (1.4)	123 (1.9)	2,171 (33.9)	6,397	

Table 1.4. Origin of blood meals obtained from *Aedes albopictus* in urban (Mercer County) and suburban (Monmouth County) habitats during 2008-2011. Percentages are provided in parentheses followed by $\pm 95\%$ CI.

Host Species	Mercer County (% [95% CI])					Monmouth County (% [95% CI])					Total
	2008	2009	2010	2011	Subtotal	2008	2009	2010	2011	Subtotal	
Human (<i>Homo sapiens</i>)	19 (45.2 [30.2-60.3])	4 (33.3 [6.7-60.0])	2 (13.3 [0.1-30.5])	12 (70.6 [48.9-92.3])	37 (43.0 [32.6-53.5])	23 (59.0 [43.5-74.4])	6 (66.7 [35.9-97.5])	5 (83.3 [53.5-99.0])	15 (60.0 [40.8-79.2])	49 (62.0 [51.3-72.7])	86 (52.1 [44.5-59.7])
Cat (<i>Felis catus</i>)	10 (23.8 [10.9-36.7])	5 (41.7 [13.8-69.6])	6 (40.0 [15.2-64.8])	3 (17.6 [0.1-35.8])	24 (27.9 [18.4-37.4])	6 (15.4 [4.1-26.7])			4 (16.0 [1.6-30.4])	10 (12.7 [5.3-20.0])	34 (20.6 [14.4-26.8])
Dog (<i>Canis familiaris</i>)	7 (16.7 [5.4-28.0])	3 (25.0 [0.5-49.5])	3 (20.0 [0.1-40.2])	1 (5.9 [0.1-17.1])	14 (16.3 [8.5-24.1])	2 (5.1 [0.1-12.1])	1 (11.1 [0.1-31.6])		2 (8.0 [0.1-18.6])	5 (6.3 [1.0-11.7])	19 (11.5 [6.6-16.4])
Virginia opossum (<i>Didelphis virginiana</i>)	2 (4.8 [0.1-11.2])		2 (13.3 [0.1-30.5])		4 (4.7 [0.2-9.1])	1 (2.6 [0.1-7.5])			2 (8.0 [0.1-18.6])	3 (3.8 [0.1-8.0])	7 (4.2 [1.2-7.3])
Gray squirrel (<i>Sciurus carolinensis</i>)	2 (4.8 [0.1-11.2])			1 (5.9 [0.1-17.1])	3 (3.5 [0.1-7.4])	2 (5.1 [0.1-12.1])			1 (4.0 [0.1-11.7])	3 (3.8 [0.1-8.0])	6 (3.6 [0.8-6.5])
Cottontail rabbit (<i>Sylvilagus floridanus</i>)							2 (22.2 [0.1-49.4])			2 (2.5 [0.1-6.0])	2 (1.2 [0.1-2.9])
White-footed mouse (<i>Peromyscus leucopus</i>)								1 (16.7 [0.1-46.5])		1 (1.3 [0.1-3.7])	1 (0.6 [0.1-1.8])
Human + Dog	1 (2.4 [0.1-7.0])		1 (6.7 [0.1-19.3])		2 (2.3 [0.1-5.5])	3 (7.7 [0.1-16.1])				3 (3.8 [0.1-8.0])	5 (3.0 [0.4-5.7])
Human + Cat	1 (2.4 [0.1-7.0])		1 (6.7 [0.1-19.3])		2 (2.3 [0.1-5.5])	1 (2.6 [0.1-7.5])			1 (4.0 [0.1-11.7])	2 (2.5 [0.1-6.0])	4 (2.4 [0.1-4.8])
Human + Deer (<i>Odocoileus virginianus</i>)						1 (2.6 [0.1-7.5])				1 (1.3 [0.1-3.7])	1 (0.6 [0.1-1.8])
Total no. identified (%)	42 (84.0)	12 (75.0)	15 (65.2)	17 (47.2)	86 (68.8)	39 (62.9)	9 (45.0)	6 (16.2)	25 (55.6)	79 (48.2)	165 (57.1)
Total no. tested	50	16	23	36	125	62	20	37	45	164	289

Table 1.5. Origin of blood meals obtained from *Culex pipiens pipiens* and *Culex restuans* in urban (Mercer County) and suburban (Monmouth County) habits during 2008–2011. Percentages are provided in parentheses followed by $\pm 95\%$ CI.

Host Species	Mercer County % [95% CI]					Monmouth County % [95% CI]					Total
	Cx. p. pipiens			Cx. restuans		Cx. p. pipiens			Cx. restuans		
	2009	2010	2011	2009	2010	2008	2011	2008	2011	Subtotal	
American robin (<i>Turdus migratorius</i>)	1 (6.7 [0.1-19.3])	10 (32.3 [15.8-48.7])	4 (26.7 [4.3-49.1])			1 (11.1 [0.1-31.6])	26 (46.4 [33.4-59.5])	1 (20.0 [0.1-55.1])	10 (47.6 [26.3-69.0])	38 (41.8 [31.6-51.9])	54 (34.0 [26.4-41.3])
Northern cardinal (<i>Cardinalis cardinalis</i>)	2 (13.3 [0.1-30.3])	6 (19.4 [5.5-33.3])	1 (6.7 [0.1-19.3])		1 (50.0 [0.1-99.0])	1 (11.1 [0.1-31.6])	5 (8.9 [1.5-16.4])	2 (40.0 [0.1-82.9])	1 (4.8 [0.1-13.9])	9 (9.9 [3.8-16.0])	19 (12.0 [6.9-17.0])
House finch (<i>Carpodacus mexicanus</i>)	4 (26.7 [4.3-49.1])	3 (9.7 [0.1-20.1])	2 (13.3 [0.1-30.3])			3 (33.3 [2.5-64.1])	5 (8.9 [1.5-16.4])		1 (4.8 [0.1-13.9])	9 (9.9 [3.8-16.0])	18 (11.3 [6.4-16.3])
European starling (<i>Sturnus vulgaris</i>)	1 (6.7 [0.1-19.3])	2 (6.5 [0.1-15.1])	2 (13.3 [0.1-30.3])	2 (50.0 [1.0-99.0])	1 (100)		5 (8.9 [1.5-16.4])		4 (19.1 [2.3-35.8])	9 (9.9 [3.8-16.0])	17 (10.7 [5.9-15.3])
Mourning Dove (<i>Zenaidura macroura</i>)		1 (3.2 [0.1-9.5])	1 (6.7 [0.1-19.3])	2 (50.0 [1.0-99.0])		2 (22.2 [0.1-49.4])	4 (7.1 [0.4-13.9])	1 (20.0 [0.1-55.1])	1 (4.8 [0.1-13.9])	8 (8.8 [3.0-14.6])	12 (7.6 [3.4-11.7])
House Sparrow (<i>Passer domesticus</i>)	3 (20.0 [0.1-40.2])	2 (6.5 [0.1-15.1])	2 (13.3 [0.1-30.3])				2 (3.6 [0.1-8.4])	1 (20.0 [0.1-55.1])		3 (3.3 [0.1-7.0])	10 (6.3 [2.5-10.1])
American crow (<i>Corvus brachyrhynchos</i>)	2 (13.3 [0.1-30.3])		1 (6.7 [0.1-19.3])				1 (1.8 [0.1-5.3])		1 (4.8 [0.1-13.9])	1 (1.1 [0.1-3.2])	4 (2.5 [0.1-5.0])
Common grackle (<i>Quiscalus quiscula</i>)							3 (5.4 [0.1-11.3])			4 (4.4 [0.2-8.6])	4 (2.5 [0.1-5.0])
Carolina chickadee (<i>Parus carolinensis</i>)	1 (6.7 [0.1-19.3])	1 (3.2 [0.1-9.5])				1 (11.1 [0.1-31.6])	1 (1.8 [0.1-5.3])		1 (4.8 [0.1-13.9])	1 (1.1 [0.1-3.2])	3 (1.9 [0.1-4.0])
Red-winged blackbird (<i>Agelaius phoeniceus</i>)									1 (4.8 [0.1-13.9])	2 (2.2 [0.1-5.2])	2 (1.3 [0.1-3.0])
Song sparrow (<i>Melospiza melodia</i>)			1 (6.7 [0.1-19.3])				1 (1.5 [0.1-4.3])				1 (0.6 [0.1-1.9])
Rock dove (<i>Columba livia</i>)			1 (6.7 [0.1-19.3])				1 (1.5 [0.1-4.3])				1 (0.6 [0.1-1.9])
Northern oriole (<i>Icterus galbula</i>)								1 (1.8 [0.1-5.3])		1 (1.1 [0.1-3.2])	1 (0.6 [0.1-1.9])
Brown thrasher (<i>Toxostoma rufum</i>)							1 (11.1 [0.1-31.6])		1 (4.8 [0.1-13.9])	1 (1.1 [0.1-3.2])	1 (0.6 [0.1-1.9])
Cedar waxwing (<i>Bombycilla cedrorum</i>)											1 (0.6 [0.1-1.9])
Yellow-crowned night heron (<i>Nyctanassa violacea</i>)											4 (2.5 [0.1-5.0])
Gray squirrel (<i>Sciurus carolinensis</i>)	1 (6.7 [0.1-19.3])	3 (9.7 [0.1-20.1])									3 (1.9 [0.1-4.0])
Cat (<i>Felis catus</i>)		1 (3.2 [0.1-9.5])					1 (1.8 [0.1-5.3])			2 (2.2 [0.1-5.2])	3 (1.9 [0.1-4.0])
Virginia opossum (<i>Didelphis virginiana</i>)		2 (6.5 [0.1-15.1])								1 (1.1 [0.1-3.2])	3 (1.9 [0.1-4.0])
Total no. identified (%)	15 (100)	31 (70.5)	15 (100)	4 (100)	1 (100)	2 (100)	9 (54.3)	56 (87.5)	21 (80.8)	91 (77.8)	159 (80.3)
Total no. tested	15	44	15	4	1	2	16	64	26	117	198

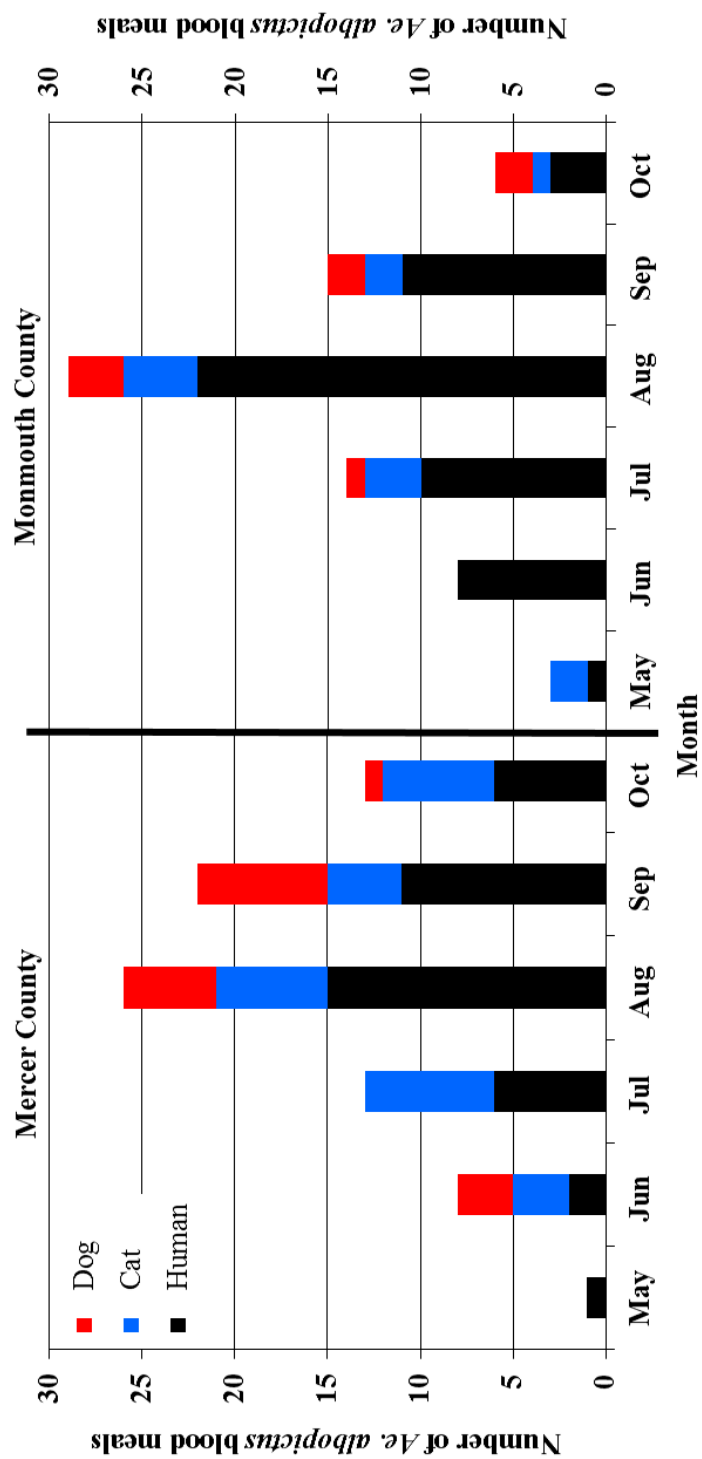


Figure 1.1. Monthly number of *Aedes albopictus*-derived blood meals from cats, dogs, and humans in urban (Mercer County) and suburban (Monmouth County) habitats of northeastern USA (2008-2011).

Chapter 2

Droplet penetration and characterization of a truck-mounted ultra-low volume mosquito adulticide spray within urban and suburban environments of New Jersey¹

Abstract

Adult control of *Aedes albopictus* via ultra-low volume is difficult because this peridomestic species occurs primarily in backyards where obstacles such as buildings and vegetation can disrupt spray plumes and droplet dispersion. We determined droplet penetration and characterization of an organophosphate adulticide applied from the ground at mid (44.54 ml ha⁻¹) and maximum (89.88 ml ha⁻¹) label rates within cryptic habitats of urban and suburban environments. Droplets were collected from all habitats, with no significant differences detected between locations within the same application rate or collection method. No differences were detected in droplet densities (mm²) between rates within urban environments, but more droplets were collected in urban (149.93 ± 11.07 SE) than suburban sites (114.37 ± 11.32) at the maximum label rate ($P = 0.003$). The excellent penetration of aerosols into cryptic habitats of an urban site was likely due to the shorter swath width afforded by our network of roads and alleys. Mid label rates displayed similar droplet density values as max label rates in urban areas, indicating that lower rates may be used effectively to reduce costs, lessen non-target effects, and increase environmental stewardship. Advances in formulations and technology are driving changes in adulticide applications, leading to use of the minimum effective dose for maximum efficacy, precision, and accountability.

¹Faraji, A., I. Unlu, T. Crepeau, S. P. Healy, S. P. Crans, G. Lizarraga, D. M. Fonseca, and R. Gaugler. 2014. Droplet penetration and characterization of an ultra-low volume mosquito adulticide spray within urban and suburban environments of northeastern USA. *Pest Manag. Sci.* *Submitted*.

Introduction

With growing globalization and commerce, mosquito invasions are increasing worldwide (Medlock et al. 2012, Rochlin et al. 2013b, Kaufman and Fonseca 2014). However, concerns for the environment and society, beckon the need to lessen the environmental impact of insecticides used to control insect vectors. Nonetheless chemical control, particularly adulticides applied as ultra-low volume (ULV) cold aerosol space sprays, remain as the only effective means of reducing transmission risk to humans during arboviral disease epidemics or when vector population densities are high (CDC 2013).

This is particularly important for the Asian tiger mosquito, *Aedes albopictus* (Skuse), which is among the most invasive and aggressive disease vectors in the world (Juliano and Philip Lounibos 2005). The range of this species is currently still expanding, particularly into highly dense human population centers in temperate urban and suburban areas, raising the public health threat of emerging and re-emerging diseases such as chikungunya and dengue (Farajollahi and Nelder 2009, Rochlin et al. 2013b). This may be particularly imminent in the case of CHIKV, as the virus is explosively spreading in the Caribbean region of the western hemisphere for the first time (Enserink 2014). The immatures of this species exploit artificial containers found in human peridomestic environments and the day-biting adults concentrate in parks and tree-lined backyards, a staple of most American cities (Unlu and Farajollahi 2012, Unlu et al. 2013). Urban mosquitoes are difficult to control because access to infested private properties is limited and the larval habitats are ubiquitous within the urban landscape. Consequently, area-

wide ULV insecticide sprays may be the only effective method to protect urban areas from *Ae. albopictus* (Farajollahi et al. 2012).

The aim of an ULV application is to deliver the most efficacious droplet size using the least amount of insecticide that will control the target mosquitoes (Mount 1998). ULV adulticide applications are conducted in the evening or early morning when a thermal inversion has occurred and light winds are present to aide in droplet carry. ULV applications have often been ineffective in controlling diurnally active urban mosquitoes, such as *Aedes aegypti* (L.) and *Ae. albopictus*. Previous researchers have hypothesized that this lack of control may be a result of resting behavior, allowing gravid or engorged females to remain sequestered during nighttime ULV applications in cryptic habitats that are sheltered from the insecticide plume (Focks et al. 1987, Perich et al. 1990, Reiter et al. 1997, Gubler 1998, Reiter 2007). Crucial information is lacking regarding penetration and density of aerosolized spray droplets within urban and suburban environments where buildings and vegetation can disrupt the movement of the spray plume. Few studies have been conducted to evaluate aerosolized droplet dynamics and characterization during real world spray applications. There is a conflicting increase in the public awareness and environmental concerns regarding insecticides versus the imminent risk to public health of an *Ae. albopictus*-driven arboviral epidemic. Consequently vector control officials must be prepared in all aspects of their integrated mosquito management (IMM) approaches to intervene with the most efficacious products and application strategies. A critical need exists for novel methods of insecticide application or new formulations to achieve successful control while maintaining environmental stewardship and accountability.

We evaluated and characterized the penetration and droplet dynamics of an ULV cold aerosol application of a novel adulticide at mid (42.7 g ha^{-1}) and maximum (86.2 g ha^{-1}) label rates within urban and suburban residential communities in temperate North America. Specifically, we were interested in determining whether the spray plume could penetrate vegetation and structural barriers to reach cryptic resting locations where diurnally active *Ae. albopictus* may be resting during a nocturnal application. We also compared the deposition efficacy of two different rotating impactors used to measure droplet volume (density) and distribution. Lastly, we compared two different techniques (digital image analysis versus traditional manual microscope readings) used to quantify droplets collected on rotating impactors.

Materials and Methods

Experimental Sites-Urban Site Selection

A highly urbanized residential field site was chosen in Mercer County, New Jersey, USA ($40^{\circ} 13' \text{ N}$, $74^{\circ} 44' \text{ W}$) as part of an area-wide management of the Asian tiger mosquito. Detailed descriptions about site selection and demographics have been published previously (Unlu et al. 2011, Farajollahi et al. 2012, Fonseca et al. 2013). The experimental field site (urban Mercer) is located in Trenton, New Jersey, in an area of low income housing (human population density of $4,286.5 \text{ /km}^2$) and consists of 48.6 ha, including 1,251 parcels with an average parcel size of $199.5 \pm 18.3 \text{ m}^2$ (Figure 2.1). Parcels correspond to a structure or house with surrounding yard, and are most often built as adjoining row homes or duplexes. Most parcels contain a sheltered alcove area between two homes, where small shrubs and trash proliferate, affording a shaded and

humid area for a cryptic resting place (Figure I.1). Our field site consists of roughly 26 residential blocks, each containing a residential street on all four sides, and divided by a drivable alley between parallel parcels (Figure 2.1). During ULV adulticide applications, both streets and alleys were driven to maximize insecticide dispersal. Within our 48.6 ha urban site, we selected five random parcels (designated as A, B, C, D, E within Figure 2.1) for use during droplet sampling. Each parcel was either part of a row home or a duplex, containing an alcove area of interest (Figure I.1). Within each parcel, we selected four stations to be used during sampling and assigned them as Front, Alcove, Porch, and Backyard (Figure 2.1). The Front and Backyard stations were closest to the line of application, since the truck-mounted sprayer drove both the street and alley. However, the Backyard station was mostly surrounded by vegetation and fencing which enclosed the yard. The Porch station was within the yard, closest to the back of the home, and the Alcove station was the most sheltered location, being completely enclosed by the front of the home and only accessible from the backyard (Figures 2.1, I.1).

Suburban Site Selection

A suburban residential field site was chosen in Monmouth County, New Jersey, USA (40° 26' N, 74° 13' W) (Unlu et al. 2011, Fonseca et al. 2013). The field site (suburban Monmouth) is located within Cliffwood Beach in the boroughs of the Raritan Bayshore (human population density of 1,907.4 /km²) and consists of 156.1 ha, including 1,247 parcels with an average parcel size of 571.1 ± 31.2 m² (Figure 2.2). Parcels in this field site are single housing structures primarily composed of middle income coastal suburban homes which are often interspersed with forest and green space remnants. This

field site consists of roughly 60 residential blocks, many of which may not include a residential street on all four sides, and none of which are divided by a drivable alley (Figure 2.2). During ULV adulticide applications, only the streets were driven to disperse the aerosolized insecticide. Within our suburban site, we also selected five random parcels (designated as A, B, C, D, E within Figure 2.2) for droplet sampling. Within each parcel, we selected four stations and assigned them as Front, Porch, Middle Yard, and Backyard (Figure 2.2). The Front station was closest to the line of application and the Backyard furthest, since the truck-mounted sprayer could only be applied from the street.

Ultra-low Volume Insecticide Application

Spray Boom Set-up and Calibrations

A Cougar[®] (Clarke Mosquito Control, Roselle, IL) cold aerosol ULV generator was used for applications. The sprayer was fitted with a SmartFlow[®] (Clarke Mosquito Control) system used in tandem with ground speed of the vehicle to ensure appropriate flow of insecticide and accurate reporting and tracking of amount of chemical used along with distance and area sprayed. The sprayer was mounted on a flatbed truck at a height of 1.8 m, and the spray boom was angled 45.5° backwards.

Droplet size and distribution are two of the most important factors affecting the success of an ULV application (Bonds 2012). Droplet size measurements were obtained for sprayer prior to applications using a DC-III[™] portable droplet measurement system (KLD Laboratories, Huntington Station, NY). For vector spraying, a droplet size range of 5 to 25 μm is most efficient, because this size is most likely to stay adrift and impinge on a mosquito and deliver a toxic dose (Haile et al. 1982). Droplet measurements are often

provided as a mass median diameter or volume mean diameter (VMD). The VMD is also provided as $Dv_{0.5}$ to represent where 50% of the spray volume or mass is contained in droplets smaller than this value. Values for a $Dv_{0.1}$ and a $Dv_{0.9}$ are also often provided to describe 10% and 90% of the cloud volume, respectively. Additionally, adulticide labels require that equipment adhere to required VMD values. We conducted two readings using the DC-III during our calibration of the Cougar ULV sprayer and acquired a $Dv_{0.1}$ value of 2.88 μm , a VMD ($Dv_{0.5}$) value of 15.18 μm , and a $Dv_{0.9}$ value of 30.82 μm . A total of 4,015 drops were counted, with only 6 droplets above 32 μm in size, and none above 48 μm .

Insecticide and ULV Application

We used a novel adulticide, DUET™ Dual-action Adulticide (Clarke Mosquito Control), which causes a benign agitation that potentially flushes mosquitoes from resting places and increasing contact with airborne droplets (Cooperband et al. 2010, Clark et al. 2013). DUET adulticide combines the pyrethroids sumithrin (5%, 44.94 g Active Ingredient L^{-1}) and prallethrin (1%, 8.99 g AI L^{-1}) with the synergist piperonyl butoxide (5%, 44.94 g AI L^{-1}). Prallethrin induces an excitatory response at sublethal concentrations and exposes mosquitoes to lethal doses of airborne sumithrin and piperonyl butoxide (Matsunaga et al. 1987, Cooperband et al. 2010). This adulticide may have advantages against not only resting gravid or engorged mosquitoes, but also diurnal mosquitoes such as *Ae. albopictus* which may be inactive during nighttime ULV applications.

The pesticide label for DUET requires ground-based spray equipment to be adjusted to deliver aerosolized droplets within a VMD of 8 to 30 μm ($D_{v0.5} < 30 \mu\text{m}$) and a $D_{v0.9}$ value of less than 50 μm . DUET was applied at a flow rate of $136.04 \text{ ml min}^{-1}$. Applications were conducted at the mid and the maximum label rates recommended on the DUET label. The mid label rate for a ground ULV application of DUET is 44.54 ml ha^{-1} , resulting in $0.40 \text{ g AI ha}^{-1}$ of prallethrin, $2.03 \text{ g AI ha}^{-1}$ of sumithrin, and $2.03 \text{ g AI ha}^{-1}$ of piperonyl butoxide. The maximum allowable label rate is 89.88 ml ha^{-1} , which delivers $0.82 \text{ g AI ha}^{-1}$ of prallethrin, $4.03 \text{ g AI ha}^{-1}$ of sumithrin, and $4.03 \text{ g AI ha}^{-1}$ of piperonyl butoxide. In urban Mercer, we conducted an application at the mid label rate and a second application at the max label rate, while in suburban Monmouth, we made a single application at the max label rate. In order to limit corruption of collection slides with other airborne pollutants (e.g., sap, dew, fuel residue, etc.) the fluorescent tracer dye Uvitex[®] OB (Ciba Corporation, Newport, DE) was mixed with the pesticide at a 0.125% weight to volume ratio, or 1.32 g L^{-1} . This dye does not alter pesticide formulations properties, droplet spectrum, or movement of pesticide droplets in the environment (Schleier III et al. 2010).

Because of the complexity and logistics involved in an area-wide metropolitan application, treatments were made at night (2:30 to 5:00 a.m.) when human activity was minimal. A single vehicle was driven at an average speed of 16.1 km h^{-1} and spray routes were designed to follow roads and alleys to maximize coverage.

Aerosol Sample Collection

Rotating Impactors

Rotating impactors are devices for collecting and measuring droplet volume and distribution. The standard impactor used in mosquito control has been the Hock™ impactor (J.W. Hock, Gainesville, FL) which uses 25 mm wide Teflon-coated microscope slides at a rotational velocity of 3.6 m sec^{-1} (Figure 2.3). However, this type of impactor is inefficient at collecting the smaller size droplets produced in adulticide applications (Bonds et al. 2009). A more robust impactor has been developed, the Florida Latham Bonds (FLB) impactor (Clayson et al. 2010), which uses 3 mm Teflon-coated acrylic rods (slides) rotating at 5.6 m sec^{-1} (Figure 2.3). In laboratory comparative assays, the FLB sampler had a higher droplet size distribution when compared to the Hock sampler across three wind speeds (1, 1.8 and 3.5 m sec^{-1}) (Bonds et al. 2009). In short, FLB impactors collect much higher densities of smaller aerosolized droplets under laboratory conditions. We deployed 20 Hock and 20 FLB impactors (Clayson et al. 2010) for our field evaluations. Each impactor uses two slides, and both impactors were placed at each station at ground level, resulting in 80 slides for measurement after each application (5 parcels x 4 stations x 2 impactors x 2 slides each).

Droplet Size and Density Determination

Slides were collected 1 h post-application and immediately placed individually inside enclosed Styrofoam coolers to limit evaporation of impinged drops. All slides were transported to the laboratory and read within 12-48 h post-application. The DropVison® - Fluorescence (Leading Edge Associates, Waynesville, NC) program is a measuring system that digitally reads slides through proprietary image analysis. The software eliminates background particles, coalesced droplets, or non-qualified drops, and only

recognizes droplets that contain the dye tracer. Slides were read by two experienced staff members under 100x microscopy. Approximately 1,000 drops from each FLB station (500 per slide), and 400 drops from each Hock station (200 per slide) were counted.

To compare data obtained through this digital approach to more standard manual analysis of droplets, we only used Hock impactors. Hock impactors traditional microscope slides and are often used by mosquito control personnel for spray plume investigations. However, manual readings of slides is labor intensive and may average >30 min for analysis of 200 drops on a single slide. We analyzed all Hock slides from urban Mercer applications conducted at the mid label and max label rates.

Meteorological Data Collection

In Mercer, meteorological data during testing was recorded for wind speed, direction, humidity, and temperature at 1 m and 10 m heights for thermal inversion observation. A Vantage Pro2™ (Davis Instruments, Hayward, CA) portable weather station was utilized during each application and set up within the treatment site 14 h prior to application and maintained until 8 h post application. A permanent weather station (KTTN) located <1 km from application site in Trenton, was used for additional meteorological data. In suburban Monmouth, meteorological data was acquired from a permanent weather station (KNJKEYPO2) located within our application site at Keyport.

Statistical Analysis

We determined droplet penetration (density) and size ($D_{v0.5}$) by analyzing ~1,000 drops from each FLB impactor (~500 drops per slide) and ~500 drops from each Hock

impactor (~250 per slide). Droplet characteristics were combined by location (Front, Alcove, Porch, Backyard) for each of the five parcels sampled to determine the mean value for each application rate and county. Differences between means were examined using two-way analysis of variance (ANOVA) with an accepted level of significance for all comparisons of $P < 0.05$ (SPSS version 18, IBM Corp, Armonk, NY).

Results

Aerosolized Spray Droplet Penetration and Characterization

Urban Mercer County

FLB Rotating Impactors

During mid label rate applications, we analyzed over 23,000 droplets from all slides, with a mean value of 1,156 drops per station and 578.1 drops per slide (Figure I.2). During max label rate applications, we analyzed over 19,600 droplets from all slides, with a mean value of 982.5 drops per station and 491.3 drops per slide (Figure I.3). We collected droplets consistently from all four stations (Front, Alcove, Porch, Backyard) with no significant differences in droplet density observed by rate ($F = 2.07$; $df = 1$; $P = 0.160$), location ($F = 0.42$; $df = 3$; $P = 0.74$), or rate within location ($F = 0.05$; $df = 3$; $P = 0.99$) (Table 2.1). Although no differences were observed in VMD ($D_{v0.5}$) values within locations at the mid label rate applications ($F = 0.14$; $df = 3$; $P = 0.93$), significant differences were observed at the max label applications between the Front-Alcove ($P = 0.02$) and Alcove-Porch ($P = 0.02$) locations (Table 2.1). Significant differences in VMD values were also observed between the mid and max label rates at the Front ($P = 0.003$) and Porch ($P = 0.003$) locations (Table 2.1).

Hock Rotating Impactors

During mid label rate applications, we analyzed over 7,800 droplets from all slides, with a mean value of 390.6 drops per station and 195.3 drops per slide (Figure I.4). During max label rate applications, we analyzed over 10,000 droplets from all slides, with a mean value of 508.9 drops per station and 254.5 drops per slide (Figure I.5). Aerosolized droplets were collected consistently from all four stations and no significant differences in droplet density were observed by location within the two application rates ($F = 0.72$; $df = 3$; $P = 0.55$). However, a significant difference in droplet density was observed between the two rates at the Front ($P = 0.002$) and Backyard ($P = 0.05$) locations (Table 2.1). Additionally, differences in VMD values were observed between the mid and max label rates at the Alcove ($P = 0.03$) and Porch ($P = 0.01$) locations (Table 2.1).

Differences between FLB and Hock Rotating Impactors

Overall, the mean droplet density (\pm SE) value obtained from FLB impactors in urban Mercer at the mid label rate was $124.37 \pm 12.45 \text{ mm}^2$ and $149.93 \pm 11.07 \text{ mm}^2$ at the max label rate, but these values were not significantly different from each other ($F = 4.70$; $df = 1$; $P = 0.06$) (Table 2.1). Droplet density obtained from Hock impactors at the mid label application rate was $4.80 \pm 0.40 \text{ mm}^2$ and $7.56 \pm 0.45 \text{ mm}^2$ at the max label rate, and again, these values were not significantly different from each other ($F = 0.06$; $df = 1$; $P = 0.82$) (Table 2.1). However, droplet density values obtained by the two rotating impactors were significantly different from each other at the mid label ($P < 0.001$) and

max label ($P < 0.001$) application rates. Additionally, the mean VMD value obtained from FLB impactors at the mid label application rate was $10.68 \pm 0.15 \mu\text{m}$ and $12.24 \pm 0.35 \mu\text{m}$ at the max label rate, which were also significantly different from each other ($P = 0.02$). The VMD mean value obtained from Hock impactors at the mid label rate was $13.36 \pm 0.76 \mu\text{m}$ and $16.43 \pm 0.3 \mu\text{m}$ at the max label rate, and again, these values were significantly different from each other ($P < 0.001$). The VMD values obtained by the two rotating impactors were also different from each other at the mid label ($P < 0.001$) and max label ($P < 0.001$) application rates.

Suburban Monmouth County

FLB and Hock Rotating Impactors

Penetration of the spray plume at the max application rate was observed on all FLB and Hock rotating impactor slides placed within all stations in suburban Monmouth (Figures I.5, I.6). We analyzed over 21,800 droplets from all FLB slides, with a mean value of 1,284.1 drops per station and 642.1 drops per slide (Figure I.6). We also analyzed over 8,300 droplets from all Hock slides, with a mean value of 490.7 drops per station and 245.3 drops per slide (Figure I.7). Spray droplets were collected from all four stations (Front, Porch, Mid Yard, Backyard) and no significant differences in droplet density were observed between the locations within each impactor type ($F = 0.23$; $df = 3$; $P = 0.88$) (Table 2.1). However, droplet density was much larger on FLB impactors and this value significantly differed between the two impactor types at each location ($P < 0.001$). Additionally, no differences were observed in VMD values between the locations

within each impactor type ($F = 0.01$; $df = 3$; $P = 0.99$) (Table 2.1). However, VMD values were larger for the Hock impactors at each location ($P < 0.001$).

Differences between Counties by Method of Collection

Since only max label rate applications were conducted in suburban Monmouth, we compared those results with the max label applications from urban Mercer. Overall, average droplet density was larger on FLB rotating impactors in urban Mercer ($149.93 \pm 11.07 \text{ mm}^2$) than in suburban Monmouth ($114.37 \pm 11.32 \text{ mm}^2$), and this difference was found to be significant ($P < 0.003$) (Figure 2.4A). The mean values for droplet density obtained from Hock impactors was $7.56 \pm 0.45 \text{ mm}^2$ in urban Mercer and $7.28 \pm 0.55 \text{ mm}^2$ in suburban Monmouth; however, no significant differences were found in droplet density gathered by Hock impactors between the counties ($P = 0.98$) (Figure 2.4A). Additionally, the VMD values obtained from FLB rotating impactors was $12.24 \pm 0.35 \text{ }\mu\text{m}$ in urban Mercer and $13.95 \pm 0.31 \text{ }\mu\text{m}$ in suburban Monmouth, which differed significantly from each other ($P = 0.002$) (Figure 2.4B). Mean VMD values obtained from Hock impactors was $16.43 \pm 0.31 \text{ }\mu\text{m}$ in urban Mercer and $18.79 \pm 0.57 \text{ }\mu\text{m}$ in suburban Monmouth, which also differed significantly from each other ($P < 0.001$) (Figure 2.4B).

Digital versus Manual Droplet Analysis for Hock Impactors

We compared digital and manual slide reading methods for only Hock impactors in Trenton at mid and max rate adulticide applications. At the mid label rate, droplet density was significantly larger ($P < 0.001$) when recorded manually ($23.10 \pm 3.60 \text{ mm}^2$)

than by the digital technique ($4.80 \pm 0.40 \text{ mm}^2$) (Figure 2.5A). This trend was also consistent at the max label rate, with a significantly ($P < 0.001$) larger droplet density recorded by the manual ($41.95 \pm 3.51 \text{ mm}^2$) than the digital ($7.56 \pm 0.45 \text{ mm}^2$) method (Figure 2.5A). Additionally, although droplet density was not significantly different between the rates within the digital method ($F = 0.60$; $df = 1$; $P = 0.44$), a difference was observed within the manual method for the rates ($P < 0.001$) (Figure 2.5A). We also observed higher VMD values at the mid label rate when comparing the digital ($13.36 \pm 0.76 \text{ }\mu\text{m}$) and manual ($10.74 \pm 0.33 \text{ }\mu\text{m}$) methods ($P < 0.001$) (Figure 2.5B). This pattern was also significant at the max label rate for the digital ($16.43 \pm 0.31 \text{ }\mu\text{m}$) and manual ($15.14 \pm 0.31 \text{ }\mu\text{m}$) methods ($P < 0.001$) (Figure 2.5B).

Meteorological Conditions

Urban Mercer County

We did not observe thermal inversions before or during the ULV applications at the mid or max label rates, which is typical for this highly urbanized environment in northeastern USA (Bache and Johnstone 1992). Temperature ($19.8 \pm 0.1 \text{ }^\circ\text{C}$) and humidity ($84 \pm 1.2 \text{ RH}$) were both stable during the mid label and also during the max label applications ($19.5 \pm 0.5 \text{ }^\circ\text{C}$ and $68.5 \pm 5.5 \text{ RH}$). Although occasional wind gusts were recorded prior to the experiment, during the ULV applications wind was absent.

Suburban Monmouth County

Meteorological data obtained from KNJKEYPO2 indicate that thermal inversions did not occur before or during the ULV applications in Monmouth County, which is also

typical for this suburban environment along the Atlantic Coast of northeastern USA. Temperature (14.5 ± 0.8 °C) and humidity (90.1 ± 4.2 RH) were both stable during the application and similar to urban Mercer, although occasional wind gusts were recorded leading up to the experiment, during the ULV application, wind was absent.

Discussion

Adulticide Efficacy on Wild Mosquito Populations and Implications for Public Health

The goal of adulticide applications is the reduction of mosquito populations. Our study did not center on the efficacy of nighttime ULV applications against the diurnally active peridomestic mosquito *Ae. albopictus*, but those results have been published beforehand (Farajollahi et al. 2012, Suman et al. 2012, Fonseca et al. 2013, 2014, Unlu et al. 2014). We have previously shown that nighttime adulticide applications do have an immediate effect on reducing populations of male and female *Ae. albopictus* within our experimental sites (Fonseca et al. 2013, Unlu et al. 2014). Although populations rebound quickly after an adulticide application due to the ubiquity of larval habitats such as disposable artificial containers and the continuous broods of emerging adults, we could extend efficacy by conducting a second adulticide application spaced one or two days apart (Farajollahi et al. 2012). We determined that dual applications at mid label rates accomplished significantly higher reduction of adults ($85.0 \pm 5.4\%$) than single full rate applications ($73.0 \pm 5.4\%$) (Farajollahi et al. 2012). Furthermore, late-season adulticide applications can provide longer relief from biting *Ae. albopictus* than earlier applications owing to the lower densities of mosquitoes and their greater vulnerability to adulticides

during these cooler periods (Fonseca et al. 2013). However, assessment of insecticide efficacy is highly dependent on appropriate droplet size, density, and penetration in order to offer the greatest probability of killing mosquitoes.

Droplet Size and Penetration of ULV Aerosols

Droplet size is a crucial factor modulating the efficiency and efficacy of aerosols generated by ULV sprayers (Bonds 2012) because it is directly related to the transport, collection effectiveness, and mortality of the intended mosquito vectors (Mount 1970). The most important requirements for an optimal droplet size are that droplets must be small enough to remain airborne, produced in sufficient density for probability of contact with flying mosquitoes, and large enough to impinge readily on the body surface of mosquitoes. The optimum droplet size for mosquito adulticiding is a VMD of 8 to 25 μm (Lofgren et al. 1973, Haile et al. 1982, Mount 1998, Bonds 2012). Our field studies consistently collected droplet sizes with a VMD ranging between $10.68 \pm 0.15 \mu\text{m}$ to $18.79 \pm 0.57 \mu\text{m}$, despite location, rate, or collection method. Additionally, these values were consistent with the pre-calibration VMD (15.18 μm) obtained from a hot-wire calibration instrument. Although differences in VMD were observed between the rates, collection methods, or locations, these differences are not operationally meaningful, as all of our VMD values were consistent with optimum droplet sizes recommended on adulticide labels and previously published reports (Mount 1970, Mount 1998, Bonds 2012).

Droplet penetration of the adulticide into sheltered habitats (such as the alcoves between duplexes or row homes) was one of the primary questions driving these

investigations. Because ULV adulticide applications are primarily conducted during the evening or nighttime, *Ae. albopictus* may be resting in natural or artificial cryptic habitats, such as alcoves, that are sheltered from the insecticide plume. Few studies have evaluated the movement of aerosols in urban habitats (Perich et al. 1992, Perich et al. 2000). Investigations into the dispersal of adulticides more frequently occur under open field or vegetative canopies, because of the simplicity of these models, and then those theories have been applied to urban habitats (Curtis and Mason 1988, Barber et al. 2007, Bonds 2012). Our study demonstrated that the aerosol plume from a truck-mounted cold aerosol application penetrates efficiently even into sheltered, cryptic habitats. Our droplet density values were consistent for all locations and no significant differences were observed between locations when using the same application rate or the method of collection. Surprisingly in urban Mercer, both rotating impactor types collected sufficient numbers of droplets even in the alcove location, which was the most sheltered of our sampling stations. Furthermore, since the adulticide was able to penetrate into these sheltered habitats, the novel excitatory component of DUET will flush mosquitoes from resting places and increase their chances of contact with airborne aerosols. The penetration of our urban adulticide application into these habitats has promising potential for vector control programs.

Droplet Density of Mid and Max Label Rate ULV Applications

We found no significant differences using the same collection method between the mean numbers of droplets collected at the two application rates. In contrast, previous authors have reported that to achieve the same efficacy in dense vegetation or urban

habitats (versus open field habitats), application rates would have to be increased several fold (Rathburn Jr and Dukes 1989, Mount 1998). However, we did not find this to be the case. We achieved the same level of penetration and droplet density at mid label rates as we did at max label rate applications. These findings are important for mosquito control programs because newly adopted federal pesticide labels severely limit the amount of active ingredients permissible per acre within a 24 h or annual period. If overall efficacy is not different at mid versus max label rates, the lower application rates should be promoted operationally, leading to reduced costs and non-target effects, and greater environmental stewardship. Sophisticated advances in formulations and technology are driving a change in ULV adulticide applications, with the ultimate goal of using the minimum effective volume of the formulated product for maximum efficacy and greater precision and accountability.

Droplet Characteristics within Urban and Suburban Habitats

The penetration of the droplets into the four stations sampled was similar within each county. However, maximum rate applications in urban Mercer displayed a significantly higher droplet density than in suburban Monmouth, as collected on the FLB impactors. This difference may be because of the smaller parcel sizes and shorter swath width (< 40 m) in urban versus suburban habitats (> 75 m), which would allow a smaller distance between the impactors and the aerosol plume as dispensed by the vehicle, increasing the probability of contact. Previous studies have determined that the most effective swath width is typically 91 to 183 m (Mount 1998). The swath widths available in suburban Monmouth are more representative of the habitats in previous investigations,

although a single study conducted in urban environments of Thailand using a swath width of 46 m found that dense housing can limit droplet penetration and density (Pant et al. 1971). In contrast, our investigations did not find a limiting factor posed by dense urban housing, but rather documented a greater droplet density within urban than in suburban habitats. The extensive network of roadways and alleys available in urban environments actually provide an advantage to truck-mounted adulticide applications by decreasing target distance. This may be an important finding because the greatest threats from mosquito vectors are in urban centers where contact between vectors and hosts are increased.

Comparison of Assay Method for Droplet Collection

Accurate sampling devices are crucial for research associated with measuring size, volume, and penetration data of mosquito control aerosols. Any sampling device used for this purpose will exhibit a collection efficiency that is a function of the device itself. However, although a number of methods are available for sampling aerosols, rotary impaction devices are gaining popularity because of their accuracy, efficiency, and ease of use (Bonds et al. 2009, Farooq et al. 2009, Clayson et al. 2010, Fritz et al. 2011). Previous studies have found that the FLB impactor collected significantly higher droplet densities as compared to the Hock sampler, (Fritz et al. 2011) and that the FLB impactor always exhibited higher collection efficiencies than the Hock impactor (Bonds et al. 2009). We also documented differences in droplet density within application rate and county when comparing the two sampling devices. The FLB rotary impactor exhibited a higher droplet density in urban Mercer at mid label, max label, and in suburban

Monmouth at max label. The Hock impactor uses standard 25 mm wide microscope slides and has a low rotational velocity when compared to the FLB impactor which uses 3 mm wide slides and has a 1.5 times higher velocity (Bonds et al. 2009, Clayson et al. 2010). The smaller surface area of the FLB slides, coupled with their faster velocity, leads to greater collection efficiencies. Our field investigations provide further evidence supporting the use of the FLB rotary impactors, particularly for sampling low-concentrations of ultra-fine aerosols relevant to vector control studies. Repeatability of field-collected data, along with accuracy and reliability of sampling methods are vital in evaluating the efficacy and droplet characteristics of insecticides.

Meteorological Conditions

Meteorology is one of the primary parameters controlling the efficacy and movement of ULV applications. Ultra-low volume adulticides dispensed for mosquito control produce a spray plume composed of ultra-fine droplets that have a low sedimentation velocity and are highly susceptible to atmospheric events (Bonds 2012). In general, gravity will pull droplets downward and a horizontal wind velocity is required to govern the longitudinal distance that the droplets will travel. Federal pesticide labels instruct that adulticide applications should only be made when wind speed is $\geq 1.6 \text{ km h}^{-1}$ and meteorological conditions are favorable for keeping the spray cloud near the ground (e.g., thermal inversion). However, we did not document any thermal inversion and all of our applications were conducted under neutral conditions, a transitory stage where no temperature gradient was recorded. Nevertheless, neutral to weakly stable conditions are considered ideal for ULV spraying (Bache and Johnstone 1992, Bonds 2012) and the lack

of convective motions may have assisted penetration and prevented our adulticide plume from ascending out of the target area. Furthermore, although the lack of wind speed was also apparent during all of our applications, this effect was not as pronounced in urban Mercer as in suburban Monmouth. Although mosquito adulticidal aerosols had penetrated equally into all sampling stations within each county, the lower droplet densities experienced in suburban Monmouth were attributed to the larger parcels and swath widths, which would have been directly influenced by the presence of greater wind speeds. Reduced wind speeds within urban settings, where a close-knit network of roadways and alleys are present, are not as important during nighttime adulticide applications when the nozzle spray velocity of the cold aerosol fogger is able to initiate movement of the droplets within habitats. These findings also hold benefit for mosquito control personnel in domestic environments where the lack of a thermal inversion and reduced wind speeds are normally experienced.

Comparison of Digital versus Manual Methods of Slide Readings

The collection of droplets on slides and their subsequent microscopic examination through manual readings by technicians to determine droplet characteristics have been widely used and accepted to assess the quality of adulticide applications (WHO 2006). However, manual readings are extremely time consuming and prone to human error, since the technician must randomly select ≥ 200 individual droplets to be measured by conducting visual sweeps across the slide surface. But the human eye will naturally navigate towards brighter, larger, or denser areas of the slide. Additionally, droplet density and size determinations must be calculated manually, potentially leading towards

additional errors. However, digital methods are gaining popularity because of their speed and accuracy (Suman et al. 2012, Farajollahi and Williams 2013). The digital method allows accurately measurement of hundreds of droplets within seconds, with an unbiased determination of VMD and density values. However, little data exists on the comparison of manual and digital methods of droplet density and size determinations. A previous study (Bonds et al. 2009) comparing the digital and manual methods found no measurement differences. Our studies comparing the digital and manual method of slide readings found a significant difference at both application rates for droplet density. In general, droplet density was much lower when determined by digital than by the manual method, and this difference was even more pronounced at the maximum rate applications. This difference could be attributed to the propensity for human readers to gravitate towards more dense areas of the slide, allowing for a quicker reading of a tedious and redundant task. Droplet size (VMD) was also significantly different during both application rates for the digital versus the manual reading methods. Although the VMD values were smaller for the manual method, these numbers were both still within the specifications of federal guidelines and pesticide label recommendations. Because the digital method can quickly measure much larger numbers of droplets and analyze a much more robust dataset, this method may provide a more accurate determination of droplet size and density. As the technology and affordability of these digital systems become more widely available, their routine use by professionals and researchers will lead to more standardized methods of droplet characteristic determinations and more meaningful comparisons between operational and research trials.

Conclusions

Droplet size, density, and penetration are crucial factors modulating the efficacy of aerosol sprays in vector control. Our experiments showed that spray droplets infiltrated all habitats sampled within our field sites, including those most sheltered from the insecticidal cloud. Mid label rates displayed similar droplet density and VMD values as max rates in urban areas, indicating that lower rates may be used effectively to reduce costs, lessen non-target effects, and increase environmental stewardship. We did not observe a limiting factor posed by dense urban housing, but rather documented a greater droplet density within urban than in suburban habitats. The shorter swath widths, availability of drivable alleys in addition to roads, and the smaller parcel sizes in urban habitats allow for a greater penetration of adulticides into target areas. Our investigations also support the use of the FLB rotary impactors, because of their efficiency in collecting low-concentrations of ultra-fine aerosols relevant to vector control studies. Repeatability of field-collected data, along with accuracy and reliability of sampling methods are vital in evaluating the efficacy and droplet characteristics of insecticides spatially and temporally. We conclude that the digital method of counting and determining droplet dynamics allows for quicker and more accurate measurements, leading to a less biased determination of VMD and density values.

Advances in formulations and technology are driving a change in adulticide applications, leading to use of the minimum effective volume for maximum efficacy and greater precision and accountability. The large and growing populations of *Ae. albopictus* in temperate urban centers make an autochthonous outbreak of an arbovirus such as chikungunya or dengue likely. This may be particularly imminent in the case of

chikungunya, as the virus is explosively spreading in the western hemisphere for the first time, having caused over 100,000 human cases in the Caribbean region in only a few months (Enserink 2014). Absent a human vaccine, we recommend that nighttime applications of ULV adulticides in areas with large populations of *Ae. albopictus* be part of an IMM approach for public health protection. Our ultimate objective is to provide vector control operators with appropriate data to base sound judgments when applying adulticides within metropolitan landscapes.

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Table 2.1. Spray plume droplet density and volume median diameter (VMD) of a mosquito adulticide applied within urban Mercer County and Suburban Monmouth County, New Jersey, USA. Sampling was conducted within four stations at mid (44.5 ml ha⁻¹) and maximum (89.9 ml ha⁻¹) label application rates using Florida-Latham Bonds (FLB) and Hock rotary impactors.

Location	Impactor type	Application rate	Unit of measure	Sampling station ^a				P
				Front	Alcove	Porch	Backyard	
Urban	FLB	44.5 ml ha ⁻¹	Droplet density	110.5 ± 33.4	115.6 ± 20.2	135.8 ± 27.0	135.6 ± 23.7	124.4 ± 12.5
		89.9 ml ha ⁻¹	(mm ² ± SE)	136.1 ± 20.9	149.7 ± 30.5	151.1 ± 24.6	162.9 ± 16.3	149.9 ± 11.1
		44.5 ml ha ⁻¹	VMD	10.5 ± 0.3	10.9 ± 0.3	10.5 ± 0.3	10.8 ± 0.3	10.7 ± 0.2
		89.9 ml ha ⁻¹	(DV _{0.5} ± SE)	12.8 ± 0.5 a	11.0 ± 1.2 b	12.9 ± 0.3 ac	12.2 ± 0.3 abc	12.2 ± 0.4
Mercer	Hock	44.5 ml ha ⁻¹	Droplet density	4.4 ± 1.1	5.0 ± 1.1	5.0 ± 0.6	4.8 ± 0.5	4.8 ± 0.4
		89.9 ml ha ⁻¹	(mm ² ± SE)	8.7 ± 1.0	6.8 ± 0.8	7.4 ± 1.3	7.4 ± 0.3	7.6 ± 0.5
		44.5 ml ha ⁻¹	VMD	13.7 ± 1.9	13.0 ± 1.0	12.2 ± 0.5	14.6 ± 2.3	13.4 ± 0.8
		89.9 ml ha ⁻¹	(DV _{0.5} ± SE)	16.5 ± 0.4	16.7 ± 0.7	16.8 ± 0.8	15.8 ± 0.6	16.4 ± 0.3
Suburban Monmouth	FLB	44.5 ml ha ⁻¹	Droplet density	131.3 ± 20.6	112.7 ± 22.3	111.6 ± 28.3	101.9 ± 24.6	114.4 ± 11.3
		89.9 ml ha ⁻¹	(mm ² ± SE)	14.6 ± 0.9	13.6 ± 0.4	14.2 ± 0.6	13.4 ± 0.7	14.0 ± 0.3
		44.5 ml ha ⁻¹	VMD	6.8 ± 1.4	8.0 ± 1.1	6.7 ± 1.2	7.9 ± 0.8	7.3 ± 0.6
		89.9 ml ha ⁻¹	(DV _{0.5} ± SE)	19.4 ± 1.5	18.4 ± 0.4	18.9 ± 1.0	18.5 ± 1.8	18.8 ± 0.6

^a All values represented for sampling stations within a row followed by the same letter are not significantly different (ANOVA, $P < 0.05$).

^b Comparison of combined means for similar units of measure within row (ANOVA, $P < 0.05$).

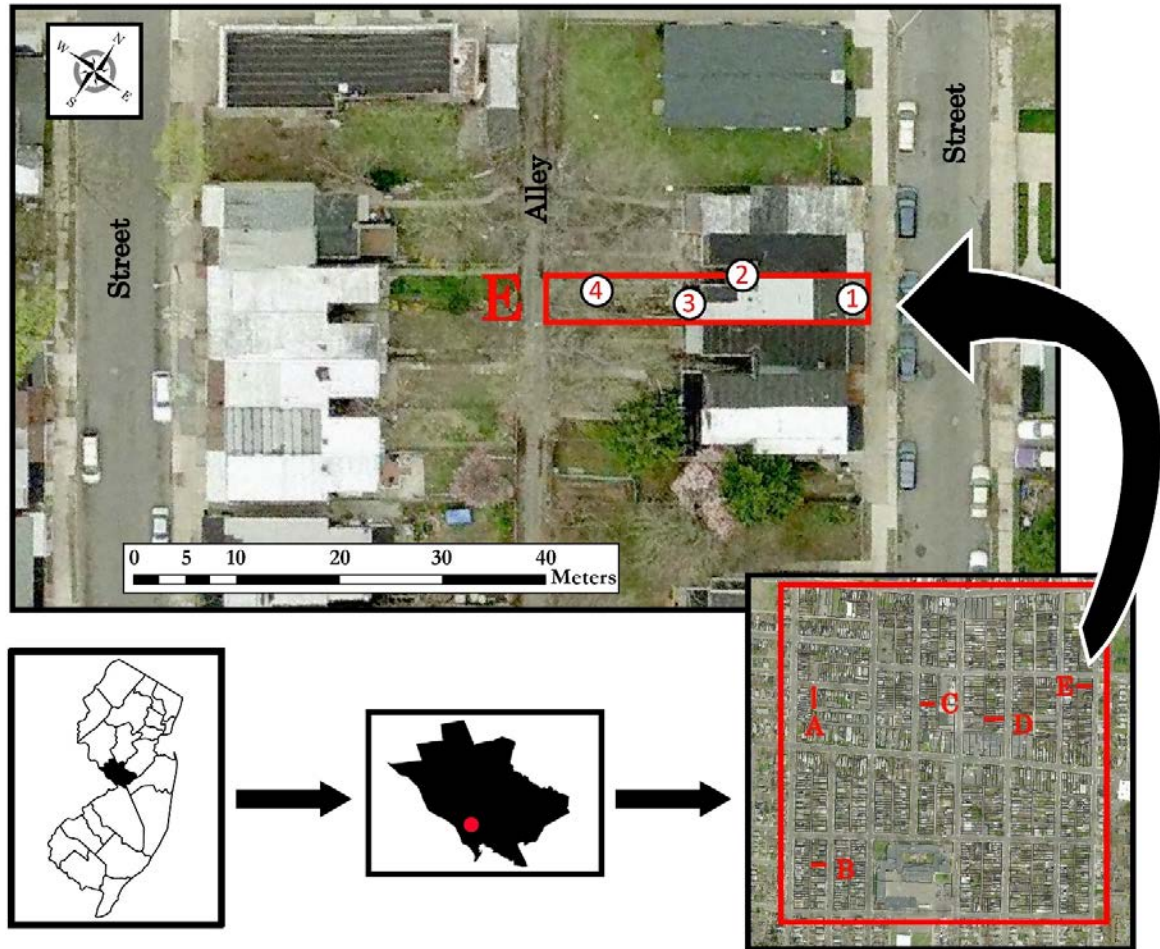


Figure 2.1. Droplet sampling locations in urban Mercer County, New Jersey, USA. Five parcels were selected within a 48.6 ha plot (A through E) and four stations were sampled within each parcel (1=Front, 2=Alcove, 3=Porch, 4=Backyard).

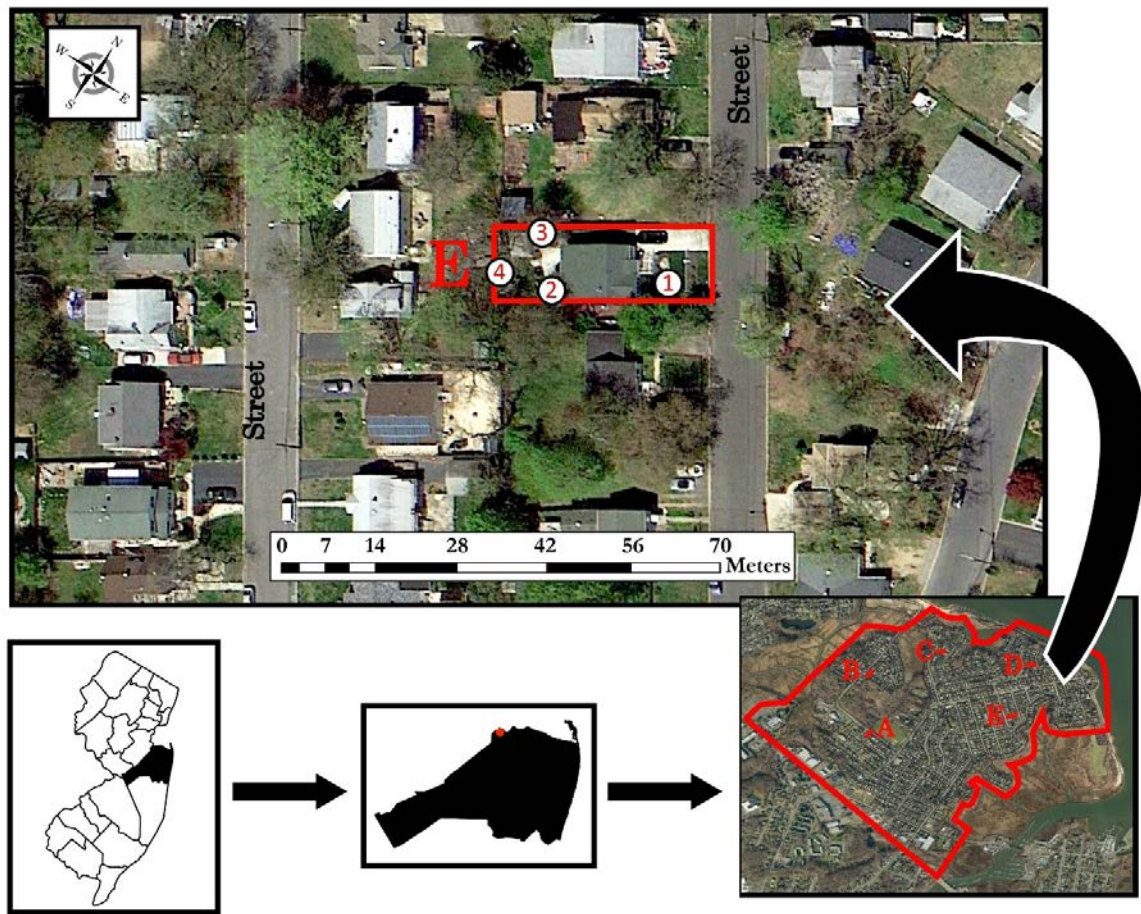


Figure 2.2. Droplet sampling locations in suburban Monmouth County, New Jersey, USA. Five parcels were selected within a 156.1 ha plot (A through E) and four stations were sampled within each parcel (1=Front, 2=Porch, 3=Mid Yard, 4=Backyard).

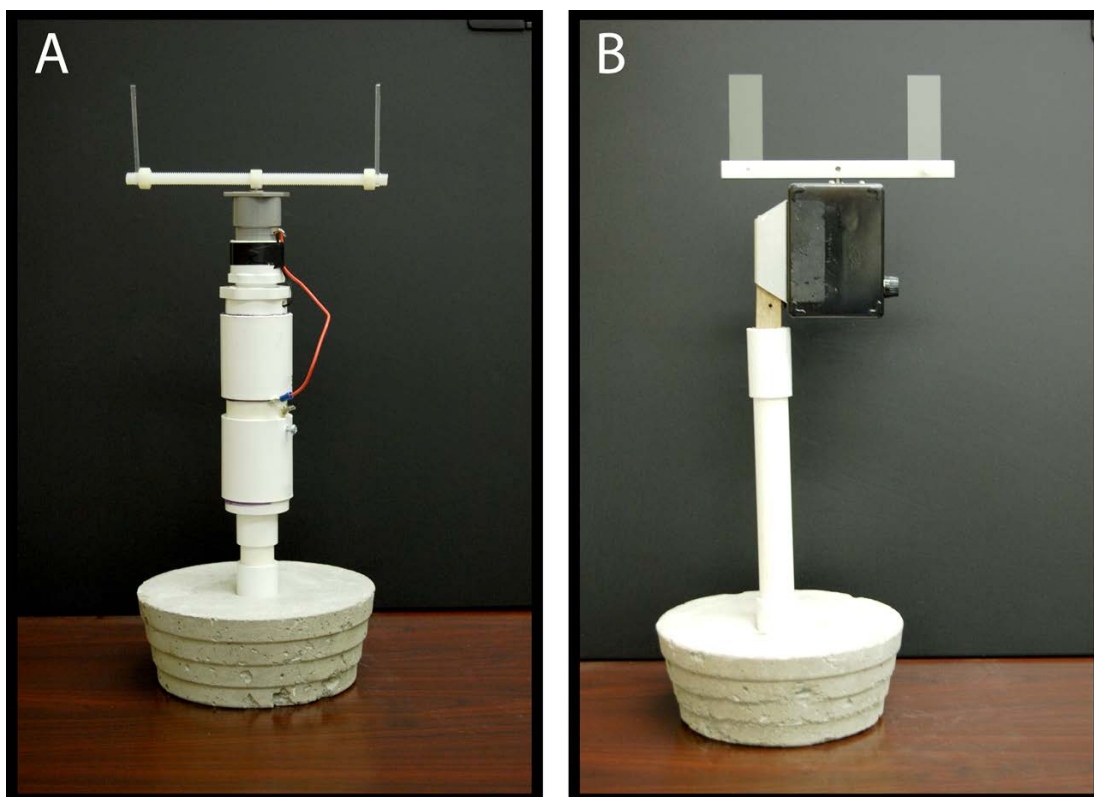


Figure 2.3. Rotating impactors used for droplet sampling of adulticidal spray plumes. A) Florida Latham Bonds (FLB) rotary-type impactor with 3 mm rods. B) Hock rotary impactor with 25 mm microscope slides.

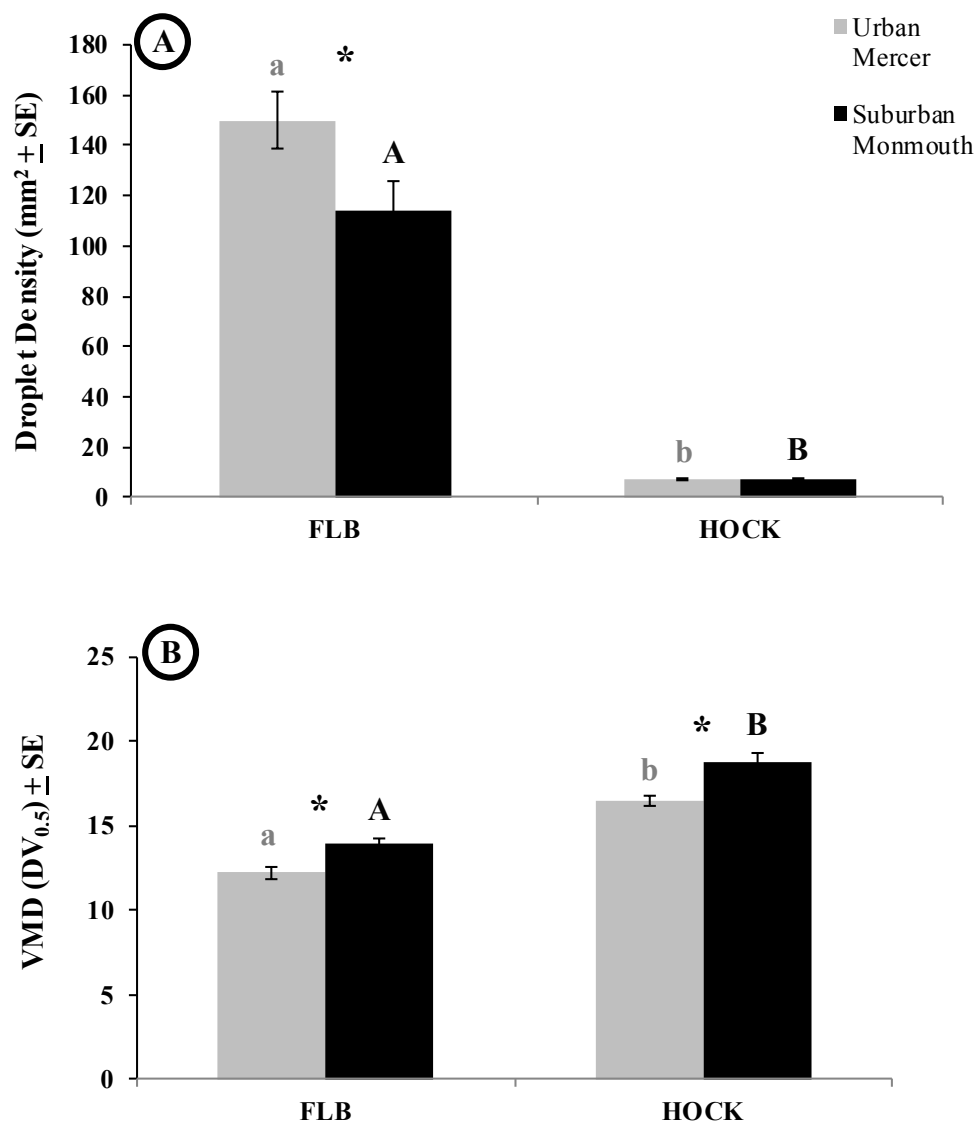


Figure 2.4. Combined mean values for droplet density and volume median diameter (VMD) of spray plume from all sampling stations in urban Mercer and suburban Monmouth sampled by both impactor types (FLB and Hock). Treatments with different letters denote significant differences within county by impactor type and asterisks denote significant differences between counties by ANOVA ($P < 0.05$).

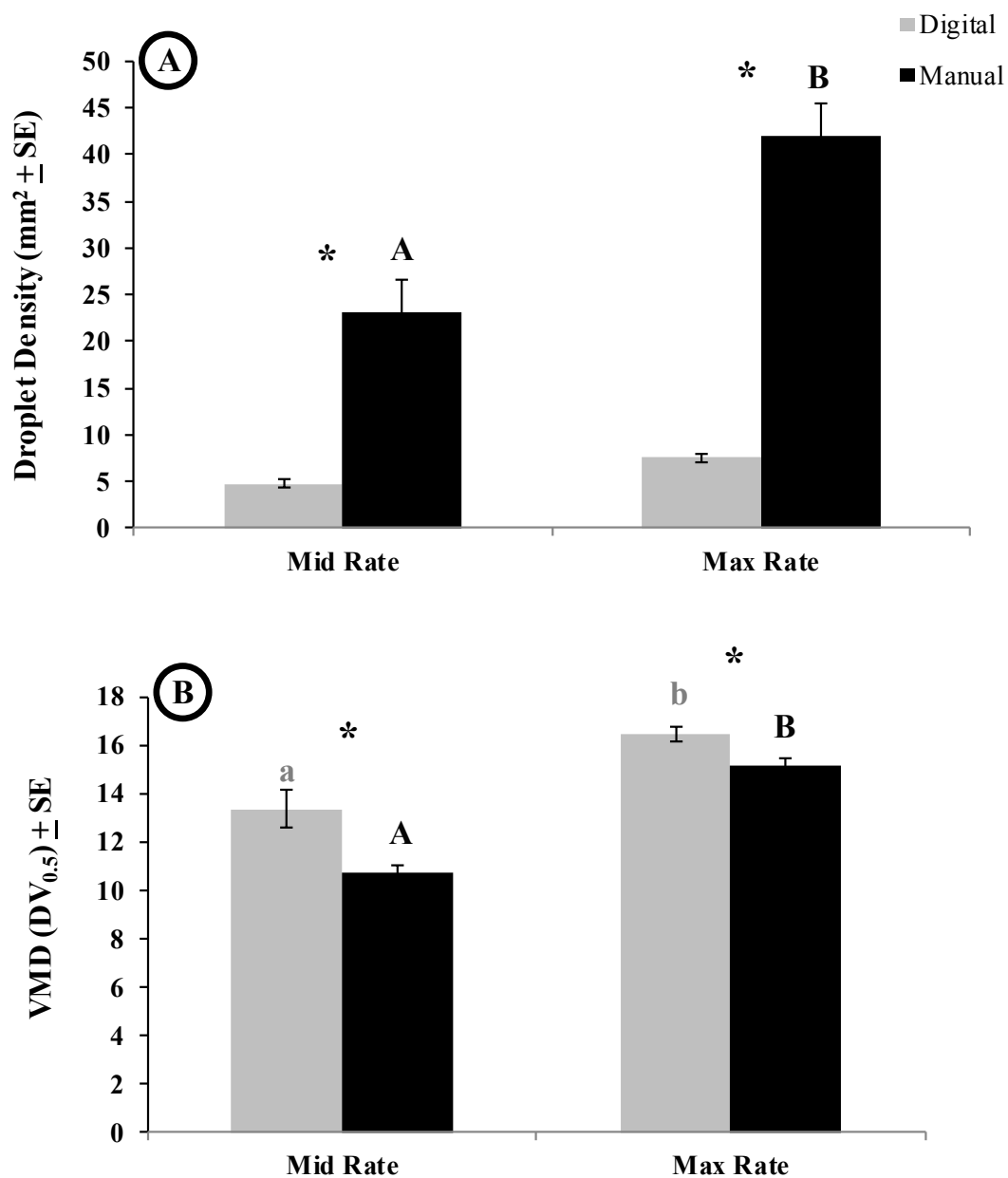


Figure 2.5. Combined droplet density and volume median diameter (VMD) of spray plume from all sampling stations in urban Mercer at two different application rates sampled by two analysis methods (digital and manual). Treatments with different letters and asterisks denote significant differences by ANOVA ($P < 0.05$).

Chapter 3

Efficacy of ultra-low volume nighttime applications of an adulticide against diurnal *Aedes albopictus* populations¹

Abstract

Aedes albopictus, the Asian tiger mosquito, continues expanding its geographic range and involvement in mosquito-borne diseases such as chikungunya and dengue. Vector control programs rarely attempt to suppress this diurnal species with an ultra-low volume (ULV) adulticide because for maximum efficacy applications are conducted at night. During 2009-2011 we performed experimental nighttime applications of a novel adulticide (DUET®) against field populations of *Ae. albopictus* within an urban site composed of approximately 1,000 parcels (home and yard) in northeastern USA. Dual applications at mid label rate of the adulticide spaced one or two days apart accomplished significantly higher control ($85.0 \pm 5.4\%$ average reduction) than single full rate applications ($73.0 \pm 5.4\%$). Our results demonstrate that nighttime ULV adulticiding is effective in reducing *Ae. albopictus* abundance and highlight its potential for use as part of integrated pest management programs and during disease epidemics when reducing human illness is of paramount importance.

¹Farajollahi, A., S. P. Healy, I. Unlu, R. Gaugler, and D. M. Fonseca. 2012. Effectiveness of ultra-low volume nighttime applications of an adulticide against diurnal *Aedes albopictus*, a critical vector of dengue and chikungunya viruses. PLoS One. 7:e49181.

Introduction

Chikungunya fever is an emerging tropical mosquito-borne disease caused by the chikungunya virus (CHIKV, genus *Alphavirus*, family *Togaviridae*) that has become widespread in the Indian Ocean region, resulting in millions of disease cases and over 250 deaths (Enserink 2007). While the acute febrile phase of the disease is usually resolved in a few days, the associated joint pain may persist indefinitely; further causing health and economic impact (Soumahoro et al. 2011). Although historically not an important vector of CHIKV, the Asian tiger mosquito, *Aedes albopictus* (Skuse) has recently emerged as the principal driver of epidemics of chikungunya (Gould et al. 2010) after a single amino acid mutation in the envelope protein of CHIKV increased its vector competence (Tsetsarkin et al. 2007, de Lamballerie et al. 2008).

Due to the widespread and increasing distribution of *Ae. albopictus* in temperate regions of North America and Europe (Benedict et al. 2007, Farajollahi and Nelder 2009, Schaffner et al. 2009) and the escalating diagnoses of cases in travelers returning from endemic or epidemic areas (Beltrame et al. 2007, Gibney et al. 2011) the risk of local CHIKV transmission in these continents is no longer conjectural, as revealed by an epidemic comprising over 200 autochthonous cases in Italy during 2007 (Rezza et al. 2007) as well as sporadic autochthonous cases in France (Gould et al. 2010). Due to the absence of a vaccine for CHIKV, mosquito control, particularly the reduction of biting populations of the primary vector *Ae. albopictus*, is the only effective means of reducing chikungunya fever cases during an epidemic.

Most federal and state guidelines for protecting the public during outbreaks of mosquito-borne diseases recommend adulticides from aircraft and truck-mounted

equipment as the most effective method of reducing transmission risk to humans (CDC 2013). These adulticide interventions are generally applied as ultra-low volume (ULV) cold aerosol sprays during night-time campaigns when a thermal inversion has occurred to keep the insecticide from dispersing upwards and light winds aid in the spread of the insecticide droplets (Mount 1998). Because prior ULV applications have not been efficacious or long lasting in controlling diurnally active urban mosquitoes, such as *Aedes aegypti* (L.) (Perich et al. 1990, Reiter 2007) and *Ae. albopictus* (Reiter et al. 1997), they have been declared ineffective in reducing dengue transmission (Gubler 1998). One reason for failure of control may be the nocturnal resting behavior of day-biting mosquitoes in natural and artificial places that are sheltered from the insecticide plume (Focks et al. 1987). The ineffectiveness of nighttime ULV applications against diurnal mosquitoes has become the conventional wisdom within the modern vector control community in the USA and many mosquito abatement programs simply do not attempt to adulticide against *Ae. albopictus* (D. Ninivaggi, personal communication). Since the public health implications of an *Ae. albopictus*-driven arboviral epidemic are great, vector control officials must be adequately prepared to intervene with efficacious application strategies and products. A critical need exists for novel methods of insecticide application or new formulations to achieve successful control.

DUET™ Dual-action Adulticide (Clarke, Roselle, IL, USA) is a new commercially available adulticide for mosquito control that causes a benign agitation [a non-biting excitation of mosquitoes] potentially flushing mosquitoes from resting places and increasing contact with airborne droplets that are more likely to impinge on flying adults (Cooperband et al. 2010). DUET adulticide combines the pyrethroids sumithrin

(5%, 44.94 g/L Active Ingredient) and prallethrin (1%, 8.99 g/L AI) with the synergist piperonyl butoxide (5%, 44.94 g/L AI). Prallethrin is reported to induce an excitatory response at sublethal concentrations and may drive mosquitoes from a resting state and expose them to lethal doses of airborne sumithrin and piperonyl butoxide (Cooperband et al. 2010). This adulticide may have advantages against not only resting gravid or engorged mosquitoes but also against diurnal mosquitoes such as *Ae. albopictus* which may be inactive during routine nighttime ULV applications by mosquito abatement programs.

The objective of this study was to evaluate the area-wide efficacy of nighttime (01:00-06:00) ground-applied ULV adulticide applications of DUET against *Ae. albopictus* within an urban residential community; we compared the abundance of *Ae. albopictus* populations within treated and untreated areas of Mercer County, New Jersey during 2009-2011. Our ultimate goal was to develop a successful ULV adulticide application strategy to be used in an integrated pest management (IPM) program for suppression of *Ae. albopictus*, both for nuisance reduction and to address imminent future outbreaks of chikungunya and dengue fever.

Materials and Methods

Study Area

During 2009, a highly urbanized residential field site was chosen in Mercer County, New Jersey, USA (40° 13' N, 74° 44' W) as part of an area-wide management of the Asian tiger mosquito (Unlu et al. 2011, Fonseca et al. 2013). The field site (Treatment Site) is located within the City of Trenton (population ~ 83,000, area 21.1 km²) and

consists of 48.4 ha, including 1,251 parcels (Figure 3.1). Parcels correspond to a structure or house with surrounding yard, and are most often built as adjoining row homes or duplexes, indicative of the type of housing in this area. Almost all adjoining parcels contain a sheltered alcove area between two homes, where vegetation and trash proliferate, affording mosquitoes a shaded and humid area for a resting place.

Additionally, socioeconomic conditions within the field site have led to a large number of abandoned homes that have been boarded shut by the City of Trenton, but often house transient humans and large amounts of trash (Unlu et al. 2011). Lack of ownership and responsibility for hygiene has increased mosquito populations within these parcels. Our field site consists of roughly 26 residential blocks, each containing a residential street on all four sides, and divided between parallel parcels by a drivable alley. During ULV adulticide applications, streets and alleys are both driven to maximize dispersal of insecticide. A second field site (40° 12' N, 74° 43' W), similar in both socioeconomic conditions and *Ae. albopictus* levels (Unlu et al. 2011), was chosen as an untreated control (Control Site), where no active interventions were performed against *Ae. albopictus*. This site consisted of 62.4 ha, including 1,064 parcels and was solely used to sample adult mosquito populations using the same protocol used in the treatment site (Fonseca et al. 2013).

Ultra-low Volume Adulticide Application

A Cougar[®] (Clarke Mosquito Control, Roselle, IL, USA) cold aerosol ULV generator was used during all adulticide applications. The unit was fitted with a SmartFlow (Clarke Mosquito Control, Roselle, IL, USA) system used in tandem with

radar ground speed of the vehicle to ensure appropriate flow of insecticide and accurate reporting and tracking of amount of chemical used along with distance and area sprayed. The sprayer was mounted in the back of a flatbed truck at a height of 1.8 m, and the spray boom was angled 45.5° pointing backwards. The vehicle was driven at an average speed of 16.1 km/h.

Droplet size measurements were obtained for the Cougar ULV machine prior to operational applications using a DC-III portable droplet measurement system (KLD Laboratories, Huntington Station, NY, USA). For vector spraying a droplet size range of 5 to 25 μm is most efficient, because this size is most likely to impinge on a mosquito and deliver a toxic dose (Haile et al. 1982). Droplet measurements for mosquito control are often provided as a mass median diameter or a volume median diameter (VMD). The VMD is also routinely provided as $D_{V0.5}$, a term used to represent a statistic where 50% of the spray volume or mass is contained in droplets smaller than this value. Most often, values for a $D_{V0.1}$ and a $D_{V0.9}$ are also provided, to describe 10% and 90% of the cloud volume, respectively. Droplet size and distribution are two of the most important factors affecting the success of an ULV application (Hoffmann et al. 2009). Additionally, adulticide labels, which are interpreted as federal law, require that given equipment adhere explicitly to required VMD values. We conducted two readings using the DC-III during our calibration of the Cougar ULV sprayer and acquired a $D_{V0.1}$ value of 2.9 μm , a VMD ($D_{V0.5}$) value of 15.2 μm , and a $D_{V0.9}$ value of 30.8 μm . A total of 4,015 drops were counted, with only 6 droplets above 32 μm in size, and none above 48 μm .

The pesticide label for DUET requires ground-based spray equipment to be adjusted to deliver aerosolized droplets within a VMD of 8 to 30 μm ($D_{V0.5} < 30 \mu\text{m}$) and

a $Dv_{0.9}$ value of less than 50 μm . For all field trials, DUET was applied at a flow rate of 136.04 ml/min. Applications during 2009 were conducted at maximum allowable label rate for a ground ULV spray (86.2 g/ha). This full label rate results in 0.81 g/ha AI of prallethrin, 4.04 g/ha AI of sumithrin, and 4.04 g/ha AI of piperonyl butoxide.

Subsequent applications during 2010-2011 were conducted at recommended mid label rate (42.7 g/ha), resulting in 0.40 g/ha AI of prallethrin, 2.02 g/ha AI of sumithrin, and 2.02 g/ha AI of piperonyl butoxide. Only single adulticide applications were conducted during 2009, however, in order to increase efficacy by compensating for gaps in coverage and missed targets, we conducted dual applications of the adulticide spaced one or two days apart during 2010 (twice) and 2011 (once). Our intention was to control adult populations with the first ULV application, wait one or two days, and conduct another adulticide application to control any newly emerged adults or mosquitoes that may have been missed with the initial application.

Truck-mounted adulticide applications were conducted at night using a single vehicle to drive the entire treatment site. Routes were designed to follow all available roads and alleys to provide maximum coverage. Each application took about 2 hours to complete and was conducted between 01:30-06:30, depending on the date of the application.

Adult Mosquito Surveillance and Analysis

Mosquitoes were sampled in our treatment site and control site on a weekly basis during 2009-2011 utilizing a network of Biogents SentinelTM (BGS) traps (Biogents AG, Regensburg, Germany). Specific details of surveillance protocols are outlined elsewhere

(Fonseca et al. 2013); but briefly, locations were chosen by overlaying a grid of specific distance intervals. We used a 175-200 m distance between BGS traps for each site.

Locations were determined using the Fishnet tool within ArcGIS Desktop 9.2

(Environmental Systems Research Institute, Redlands, CA, USA). These distances were based on current knowledge of *Ae. albopictus* flight range (Estrada-Franco and Craig 1995) and the available resources within each site. Two hundred meter sampling resulted in 12 traps within the treatment site and 15 traps within the control site during 2009-2010, while 175 meter sampling resulted in 16 traps within the treatment site and 24 traps within the control site during 2011. Sampling was performed with BGS traps deployed weekly for 24 hours and deployed in backyards (near vegetation or shade) of each parcel selected. Each week, traps were placed in the same location within the backyards.

Permissions to place BGS traps within each parcel were acquired at the beginning of each season from individual property owners. The BGS trap was used with a solid BG-lure (Biogents AG, Regensburg, Germany) containing ammonia, lactic acid and fatty acids, components known to be particularly attractive to *Ae. albopictus* (Farajollahi et al. 2009).

Mosquitoes recovered from traps were placed in containers and transported to the laboratory on dry ice for identification and pooling. We calculated the mean number of *Ae. albopictus* adults (male+female) collected during each sampling session in BGS traps within each site. Adulticide applications were performed when environmentally, logistically, and operationally feasible within the treatment site when a threshold mean of ≥ 5 *Ae. albopictus* (male+female) adults were detected in our weekly BGS surveillance. This number was chosen because 3 bites have been reported as a common nuisance threshold driving residents indoors (Read et al. 1994), and an average of 5 bites/day by

Ae. albopictus in Italy has been recorded as intolerable (Carrieri et al. 2008). Percent control after ULV application of adulticides was calculated by using an algebraic variation of Henderson's method (Henderson and Tilton 1955) using the formula: percent control = $100 - [(T/U)100]$, where T is the post application mean divided by the pre application mean in the treatment site and U is the post application mean divided by the pre application mean in the control (no intervention) site. Although additional integrated pest management intervention efforts such as education, source reduction, and application of larvicides were being conducted within our treatment site as part of a larger project (Fonseca et al. 2013), none would have an immediate effect on adult populations. Thus, our analyses concentrated on the overnight percent reduction of adult populations. We used ANOVA (JMP 8, SAS Institute, Cary, NC, USA) to examine the efficacy of a single ULV application versus a dual application, and full label rate versus mid label rate. Percentages were arcsin transformed prior to analysis (Sokal and Rohlf 1981). No specific permits were required for the collection of adult mosquitoes or the described field studies, which were developed with homeowners assent by professional county mosquito control personnel. These studies did not involve endangered or protected species.

Meteorological Data Collection

During each application, meteorological data was recorded for wind speed, direction, humidity, and temperature at 1 m and 10 m heights for thermal inversion observation. A Vantage Pro2 (Davis Instruments, Hayward, CA, USA) portable weather station was set up within the treatment site 2 hours prior to application and maintained

until 2 hours post application. Additional meteorological data was obtained from a permanent weather station located at Trenton-Mercer Airport, situated 7.5 km from the application site.

Results

The experiments were performed during the 2009, 2010, and 2011 active seasons for *Ae. albopictus*. Adulticide applications were conducted in unison with an intensive surveillance program and were one of the components of an IPM strategy being developed for control of *Ae. albopictus*. We conducted our first application of DUET at full label rate and then proceeded to evaluate mid label rate applications in different combinations (Table 3.1). Although most applications of adulticide were initiated when the mean number of adults (male+female) captured in BGS traps were above 5, on one occasion we started with lower numbers (4.1 ± 1.4) because we were testing the effect of adulticiding on populations of *Ae. albopictus* at the end of the season. Although evaluating the efficacy of control measures may be more difficult when adult numbers are already low, this test yielded control levels similar to those at other mid label rate single applications (Table 3.1). As a result, the removal of this treatment from the analysis does not affect the overall results (data not shown). The number of post-treatment adults was measured for 24 hrs starting the afternoon of the day (night) when treatment occurred. For duplicate treatments, the post-treatment counts were made after the second treatment only. In all cases post-treatment values were lower than 5 (2.3 ± 0.7). The absence of significant wind was a constant (Table 3.1) as well as high humidity and

air temperatures at night in the mid 20°C range, which are characteristic of urban areas in mid-Atlantic states during the summer months (Bache and Johnstone 1992).

We found that single ULV adulticide applications at the full label rate of 86.2 gm/ha resulted in a percent reduction of $72.7 \pm 5.4\%$ (SE), which is significantly higher [$p = 0.04$] than single ULV applications at the mid label rate of 42.7 gm/ha ($54.0 \pm 4.7\%$). However, dual applications at mid label rate were significantly more effective ($p = 0.003$) than single applications at full rate and resulted in an average percent reduction of $85.0 \pm 5.4\%$. Dual applications at the full label rate could not be conducted without exceeding label guidelines. Overall the two variables, application rate (full versus mid) and application type (single versus dual), explained 75% of the variance in percent control ($R^2=0.75$).

Discussion

Evaluating the efficacy of aerosol sprays for adult mosquito control is critical to assessing their suitability, especially during epidemics when fast reduction in populations of biting females is paramount. Over three years and multiple nighttime adulticide applications, we observed an overall significant average percent reduction in adult populations of day-biting *Ae. albopictus* mosquitoes as measured using BGS trap surveillance. Our results provide direct evidence that nighttime applications of an ULV adulticide are effective in reducing *Ae. albopictus* abundance.

Our measures of adult population reductions were derived from BGS traps, a relatively new sampling device for capturing container-inhabiting *Aedes* mosquitoes. The BGS trap has been proven as an effective alternative to other collection devices and traps

such as backpack aspirators, gravid traps, variations of carbon dioxide-baited traps, and the Fay-Price trap (Williams et al. 2006, Farajollahi et al. 2009, Obenauer et al. 2010) for obtaining estimates of field abundance of *Ae. albopictus*, and approximates human landing rate estimates (Kroeckel et al. 2006, Obenauer et al. 2010). By targeting adult mosquitoes, BGS traps provide an actual estimate of the biting populations, and hold an immediate advantage over other sampling and population assessment methods (e.g. Breteau, container, house indices or pupae per person) which are relatively more labor intensive and plagued with levels of assumptions, imprecision, and unpredictability (Focks et al. 2000). BGS traps provide an opportunity for improved adult entomological surveillance and have been used successfully as a rapid response tool for detection of *Ae. albopictus* (Ritchie et al. 2006) and to gauge efficacy of various control measures targeted against this species (Fonseca et al. 2013). Furthermore, we utilized not only before/after numbers, but also comparisons between treated and untreated sites to determine the immediate percent reduction effects of adulticide applications on populations of *Ae. albopictus* in temperate North America.

Significantly, we found a greater effect on adult *Ae. albopictus* populations through utilization of dual or repeated applications of adulticide at mid label rate. Previous studies have indicated that two adulticide treatments using dieldrin (a chlorinated hydrocarbon similar to DDT which is now banned in most of the world) as a thermal fog during the day and spaced a week apart, increased and prolonged control of *Ae. albopictus* for up to eight weeks (Dowling 1955). Adulticide interventions by aircraft during the day against *Ae. aegypti* using malathion applied twice 4 days apart have also shown upwards of 90% control for over 10 days post application (Lofgren et al. 1970).

We conducted dual ULV applications of adulticide at mid label rate resulting in an average reduction of 85% in *Ae. albopictus*. Furthermore, although previous studies have indicated that ULV adulticides need to be applied at maximum rate (Mount 1998, Barber et al. 2007), we found that even mid label rate applications of the insecticide had a significant effect on *Ae. albopictus*. Our field applications were conducted in a highly urbanized area in which we were able to drive both roadways and alleys to further enhance penetration of product and contact with mosquitoes. This finding has promising potential for vector control programs that are often under scrutiny about pesticide costs and also usage/exposure from the general public and must face increasing regulations and adulticide amount limits from local/federal government.

The rationale for adulticiding during epizootics or epidemics of arboviruses is to reduce the number of infected mosquitoes and thus interrupt pathogen transmission. Studies of *Ae. aegypti* following ULV adulticide applications have shown that only 8% of female mosquitoes dissected post-treatment were parous, as compared with parity rates of 30% in the pre-treatment area and 40% in an untreated area (Lofgren et al. 1970). The reduction in parous females, which are most likely to be infected, makes ULV adulticiding a very important component of a comprehensive intervention program geared towards protection of public health from mosquito-borne diseases. Careful examination of the 2007 outbreak of chikungunya fever in Italy, the first large outbreak in a temperate climate region, indicates that a larger epidemic was thwarted by timely control interventions (Poletti et al. 2011). Although it is still debated what level of reduction in adult populations is necessary and sufficient to prevent disease outbreaks, transmission models developed for *Ae. aegypti* and dengue suggest that the degree of

suppression required to eliminate summertime spread of the disease may be lower than 83% in some cases but closer to 90% in others (Focks et al. 2000, Strickman and Kittayapong 2003). The reduction in *Ae. albopictus* abundance we achieved through nighttime adulticiding (85%) would likely result in a decrease in the number of infective bites received by the human population and would consequently impact the transmission of an arbovirus such as dengue or CHIKV.

In conclusion we provide evidence that a nighttime ULV application of a synthetic pyrethroid is efficacious in reducing the abundance of *Ae. albopictus* in an urban environment and that dual applications using mid label rates, spaced one or two days apart, provide levels of reduction in the adult populations of *Ae. albopictus* in the upper range of which is necessary for interruption of arboviral transmission. The large and growing populations of *Ae. albopictus* in several northeastern urban centers such as Washington (DC), Philadelphia, Trenton, and New York City (Benedict et al. 2007, Farajollahi and Nelder 2009, Rochlin et al. 2013b) make a large autochthonous outbreak of an arbovirus such as CHIKV or dengue a clear and present danger. We recommend that nighttime applications of ULV adulticides in areas with large populations of *Ae. albopictus* be considered as part of an integrated mosquito management approach for public health protection.

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Table 3.1. Summary of adulticide applications and BGS trap results during 2009-2011, Mercer County, New Jersey.

Year	Application Date	Application Time (am)	Application Rate (gm/ha)	Temperature (°C)	Relative Humidity (RH %)	Wind Speed (km/h)	Treatment Site		Control Site		Percent Control *
							Pre-treatment Mean (\pm SE)	Post-treatment Mean (\pm SE)	Pre-treatment Mean (\pm SE)	Post-treatment Mean (\pm SE)	
2009	05-Aug-09	02:45 to 05:00	86.2	22.2	94%	<1.6†	8.3 \pm 1.97	2.0 \pm 0.66	27.1 \pm 5.3	16.5 \pm 2.81	61%
	19-Aug-09	03:15 to 06:00	86.2	22.8	93%	5.6	14.3 \pm 3.92	4.1 \pm 0.88	18.1 \pm 4.74	21.4 \pm 4.99	74%
	16-Sep-09	03:00 to 05:00	86.2	19.2	76%	10.5	7.8 \pm 2.07	1.1 \pm 0.36	19.7 \pm 3.87	16.3 \pm 3.77	83%
2010	29-Jul-10	04:00 to 05:30		25.3	85%	8.1					
	&		42.7	&	&	&	10.8 \pm 2.89	2.6 \pm 0.71	9.1 \pm 1.68	13.9 \pm 3.56	82%
	30-Jul-10‡	04:00 to 06:00		18.9	65%	9.9					
	18-Aug-10	05:00 to 06:30		22.2	69%	<1.6					
	&		42.7	&	&	&	10.5 \pm 2.30	0.9 \pm 0.32	14.2 \pm 2.78	11.4 \pm 1.36	90%
2011	20-Aug-10‡	05:30 to 07:00		21.1	73%	<1.6					
	02-Sep-10	05:00 to 06:00	42.7	22.8	73%	<1.6	5.7 \pm 1.22	3.1 \pm 1.01	12.1 \pm 2.3	11.5 \pm 1.74	43%
	4-Aug-11	01:45 to 03:25		20.6	87%	5.6					
	&		42.7	&	&	&	6.6 \pm 1.24	1.6 \pm 0.63	10.3 \pm 1.57	14.9 \pm 1.76	83%
	5-Aug-11‡	03:30 to 05:00		22.2	95%	<1.6					
2011	14-Sep-11	02:30 to 05:00	42.7	21.1	87%	3.2	15.5 \pm 2.80	3.2 \pm 1.32	26.3 \pm 5.02	17.3 \pm 3.41	68%
	27-Sep-11	02:30 to 04:00	42.7	21.7	93%	<1.6	4.1 \pm 1.44	2.5 \pm 0.67	12.7 \pm 2.80	16.4 \pm 3.95	54%

*Percent control following Henderson's equation: $100 - ((T/U)^{100})$.

†Wind speeds of <1.6 km/h indicate that wind was negligible during ULV application.

‡Denotes a tandem application of adulticide.



Figure 3.1. Map of ULV adulticide treatment site in Mercer County, New Jersey, USA, 2009-2011. Inset of Mercer County in the top left displays locations of treatment and no intervention sites, and detailed map below displays locations of BGS traps, parcels, and roads/alleys within only the treatment site.

Conclusions

All good research should start with a significant endeavor and in the end, inevitably lead to further questions. This is not to say that the work was done in vain, but that any information gathered on the intricacies of nature and the interrelatedness of the various disciplines will further advance our understandings on the complexity of living organisms and ultimately lead to improved public health measures. My investigations sought to provide further knowledge on the biology and control measures aimed at the Asian tiger mosquito, *Aedes albopictus* (Skuse), in northeastern USA.

To understand the role of *Ae. albopictus* in endemic and exotic disease ecology and assess the public health threat of an introduced arbovirus outbreak, I investigated the host feeding patterns of this species in the northernmost limit of its geographic range in North America. I found that the blood feeding behavior of field-collected *Ae. albopictus* establish that this species is primarily mammalophilic in peridomestic environments of northeastern USA, with over 90% of their blood meals derived from humans and domesticated pets.

However, unlike some previous studies, I did not document avian-derived blood meals in any of my *Ae. albopictus* samples despite extensive testing with avian-specific primers. My findings cannot be attributed to the method of collection, blood meal identification methodology, host availability, or spatial/temporal factors, since the *Culex* mosquitoes collected in the same traps at the same time, were found to feed predominately on birds within my study sites as expected (Apperson et al. 2004, Molaei et al. 2006, Molaei et al. 2008). The lack of blood meals obtained from birds by *Ae. albopictus* suggest that this species may have limited exposure to endemic avian

arboviruses, such as West Nile virus, which is supported by the lack of virus isolations in over 34,500 specimens assayed in a complementary study (Armstrong et al. 2013).

However, the high mammalian affinity of *Ae. albopictus* suggests that this species may be an efficient vector of mammal-driven zoonoses such as La Crosse virus, and human-driven anthroponoses such as dengue and chikungunya.

Large proportions of human-derived blood meals have been documented previously in *Ae. albopictus* and a few studies have reported that field populations feed exclusively on humans (Ponlawat and Harrington 2005, Dennett et al. 2007, Kim et al. 2009, Muñoz et al. 2011), but the use of aspirators and human bait may bias these estimates. Additionally, recent investigations in temperate Italy have shown that *Ae. albopictus* feeding patterns differ between urban and rural habitats, with 90% of blood meals in urban areas from humans and only 20% being human-derived in rural habitats (Valerio et al. 2010). But my results report a significantly higher proportion of human blood meals in *Ae. albopictus* from suburban areas, rather than the densely populated urban areas. This was surprising, because of the higher (>2 times) human population density in urban Mercer County. However, suburban dwellers often spend more time outdoors gardening or undertaking leisure activities in backyards during daylight hours which will increase their exposure to diurnal mosquitoes. In addition, proportions of *Ae. albopictus* feeding on cats and dogs was higher in urban than suburban sites, likely reflecting large populations of feral cats in urban low income areas (Gehrt et al. 2013) and the fact that often dogs are kept in outside cages or yards for homeowner protection (Unlu and Farajollahi 2012). In contrast, suburban residents primarily keep their pets indoors and availability of these hosts for *Ae. albopictus* may be reduced. The

significantly greater anthropophagic behavior of *Ae. albopictus* in more affluent suburban, versus low-income urban habitats of northeastern USA indicates that a larger public health concern may exist within suburban landscapes, despite lower human population densities. Higher proportions of *Ae. albopictus* feeding on cats and dogs within urban environs may help fuel local mosquito populations but it may also afford zooprophyllaxis protection for humans during epidemic outbreaks of anthroponoses such as dengue or chikungunya, because it will divert vector feeding to non-susceptible dead-end hosts.

However, growing populations of *Ae. albopictus* in major metropolitan urban and suburban centers, make a large autochthonous outbreak of an arbovirus such as chikungunya or dengue viruses a clear and present danger. I also cannot rule out the possibility that *Ae. albopictus* may occasionally act as a bridge vector for endemic pathogens such as St. Louis encephalitis virus and West Nile virus by feeding on infected hosts when their abundance is great. Given the difficulty in successful suppression of *Ae. albopictus* in areas where it has become firmly established (Fonseca et al. 2013, Rochlin et al. 2013b), I strongly recommend further ecological investigations on this species and caution public health practitioners and policy makers to install proactive measures for the imminent mitigation of an exotic pathogen outbreak.

In regards to determining the utility of a truck-mounted cold aerosol ultra-low volume (ULV) adulticide within urban and suburban environments, I investigated the penetration and characteristics of aerosol sprays into cryptic habitats where buildings and vegetation can disrupt spray plumes. I found that spray droplets infiltrated all habitats sampled within my field sites, including those most sheltered from the insecticidal cloud.

Because ULV adulticide applications are primarily conducted during the evening or nighttime, *Ae. albopictus* may be resting in natural or artificial cryptic habitats, such as alcoves, that are sheltered from the insecticide plume. Few studies have evaluated the movement of aerosols in urban habitats (Perich et al. 1992, Perich et al. 2000).

Investigations into the dispersal of adulticides more frequently occur under open field or vegetative canopies, because of the simplicity of these models, and then those theories are applied to urban habitats (Curtis and Mason 1988, Barber et al. 2007, Bonds 2012). I did not observe a limiting factor posed by dense urban housing, but rather documented a greater droplet density within urban than in suburban habitats. The shorter swath widths, availability of drivable alleys in addition to roads, and the smaller parcel sizes in urban habitats allow for a greater penetration of adulticides into target areas. My investigations demonstrate that the spray plume from a truck-mounted cold aerosol application penetrates efficiently even into sheltered, cryptic habitats. The droplet density values were consistent for all locations and no significant differences were observed between locations when using the same application rate or the method of collection. Surprisingly in urban Mercer, both rotating impactor types collected sufficient numbers of droplets even in the alcove location, which was the most sheltered of my sampling stations. Furthermore, since the adulticide was able to penetrate into these sheltered habitats, the novel excitatory component of new adulticides will flush mosquitoes from resting places and increase their chances of contact with more toxic airborne aerosols (Cooperband et al. 2010).

I also found that mid label rates displayed similar droplet density values as maximum application rates in urban areas, indicating that lower rates may be used

effectively to reduce costs, lessen non-target effects, and increase environmental stewardship. My investigations also support the use of newly available rotary impactors and droplet counting software, because of their efficiency in collecting and reading low-concentrations of ultra-fine aerosols relevant to vector control studies. Repeatability of field-collected data, along with accuracy and reliability of sampling methods are vital in evaluating the efficacy and droplet characteristics of insecticides spatially and temporally. The penetration of an urban adulticide application into cryptic habitats and the similarities between mid and maximum label application rates has promising potential for vector control programs.

With respect to determining the efficacy of nighttime ULV adulticides in peridomestic environments, I investigated the impact (reduction) against diurnal biting populations of *Ae. albopictus* using two different application rates and methods. I found that dual adulticide applications spaced one or two days apart, at mid label rates were significantly more effective than single applications at full rate. The overall percent reduction for these dual applications was about 85%. However single adulticide applications at the full label rate resulted in a higher percent reduction (73%) than single adulticide applications at the mid label rate (54%).

Furthermore, although previous studies have indicated that ULV adulticides need to be applied at maximum rate (Mount 1998, Barber et al. 2007), I found that even mid label rate applications of the insecticide had a significant effect on *Ae. albopictus*. My field applications were conducted in a highly urbanized area in which I was able to drive both roadways and alleys to further enhance penetration of product and contact with mosquitoes. This finding has encouraging potential for vector control programs that are

often under scrutiny about pesticide costs and also usage/exposure from the general public and must face increasing regulations and adulticide amount limits from local/federal government.

The rationale for adulticiding during epizootics or epidemics of arboviruses is to reduce the number of infected mosquitoes and thus interrupt pathogen transmission. Studies on *Aedes aegypti* L. following ULV adulticide applications have shown that only 8% of female mosquitoes dissected post-treatment were parous, as compared with parity rates of 30% in the pre-treatment area and 40% in an untreated area (Lofgren et al. 1970). The reduction in parous females, which are most likely to be infected, makes ULV adulticiding a very important component of a comprehensive intervention program geared towards protection of public health from mosquito-borne diseases. Careful examination of the 2007 outbreak of chikungunya fever in Italy, the first large outbreak in a temperate climate region, indicates that a larger epidemic was thwarted by timely control interventions (Poletti et al. 2011). Although it is still debated what level of reduction in adult populations is necessary and sufficient to prevent disease outbreaks, transmission models developed for *Ae. aegypti* and dengue suggest that the degree of suppression required to eliminate summertime spread of the disease may be lower than 83% in some cases but closer to 90% in others (Focks et al. 2000, Strickman and Kittayapong 2003). The reduction in *Ae. albopictus* abundance I achieved through nighttime adulticiding (85%) would likely result in a decrease in the number of infective bites received by the human population and would consequently impact the transmission of an arbovirus such as dengue or chikungunya.

In conclusion I provide evidence that a nighttime ULV application of a synthetic pyrethroid is efficacious in reducing the abundance of *Ae. albopictus* in an urban environment and that dual applications using mid label rates, spaced one or two days apart, provide levels of reduction in the adult populations of *Ae. albopictus* in the upper range of which is necessary for interruption of arboviral transmission. The large and growing populations of *Ae. albopictus* in several northeastern urban centers make a large autochthonous outbreak of an arbovirus such as chikungunya or dengue imminent. I recommend that nighttime applications of ULV adulticides in areas with large populations of *Ae. albopictus* be considered as part of an integrated mosquito management approach for public health protection.

Appendix I

Aerosol Penetration and Characteristic Figures

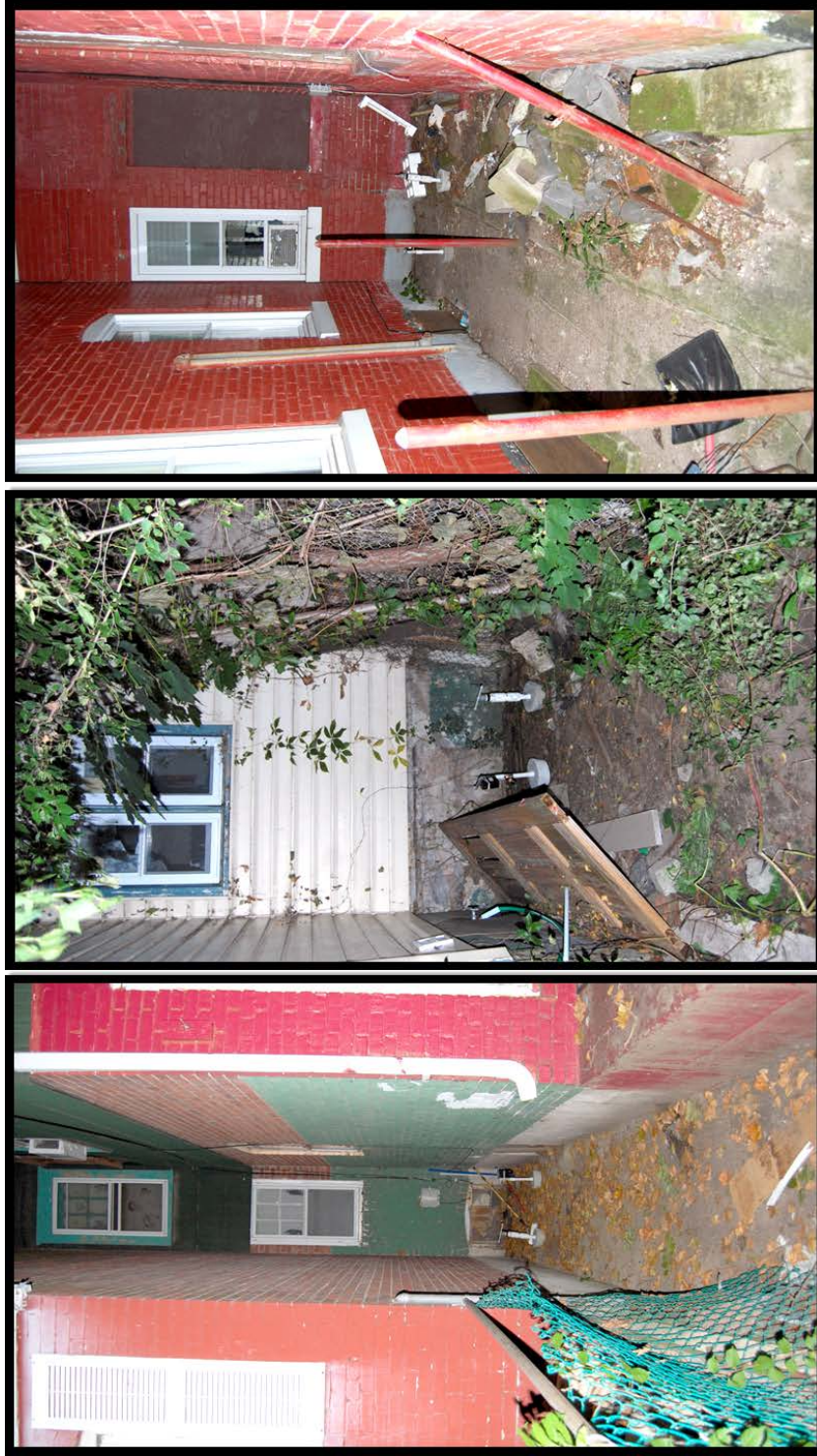


Figure I.1. Three representative sheltered alcove stations between two adjoining parcels (homes) in urban habitats of our sampling sites.

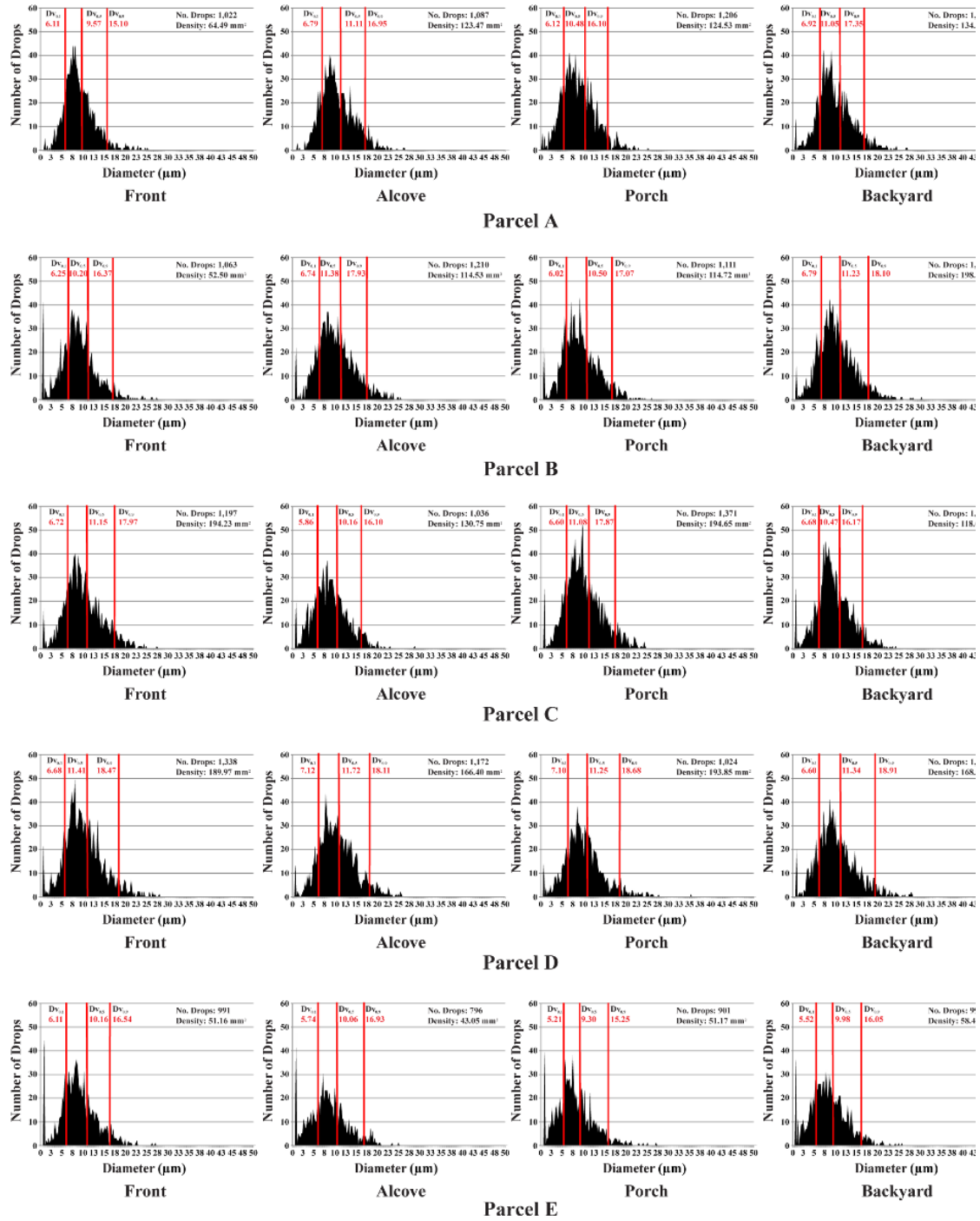


Figure I.2. Droplet characteristics of a mid label ULV adulticide application within individual stations and parcels in urban Mercer as sampled by FLB type impactors.

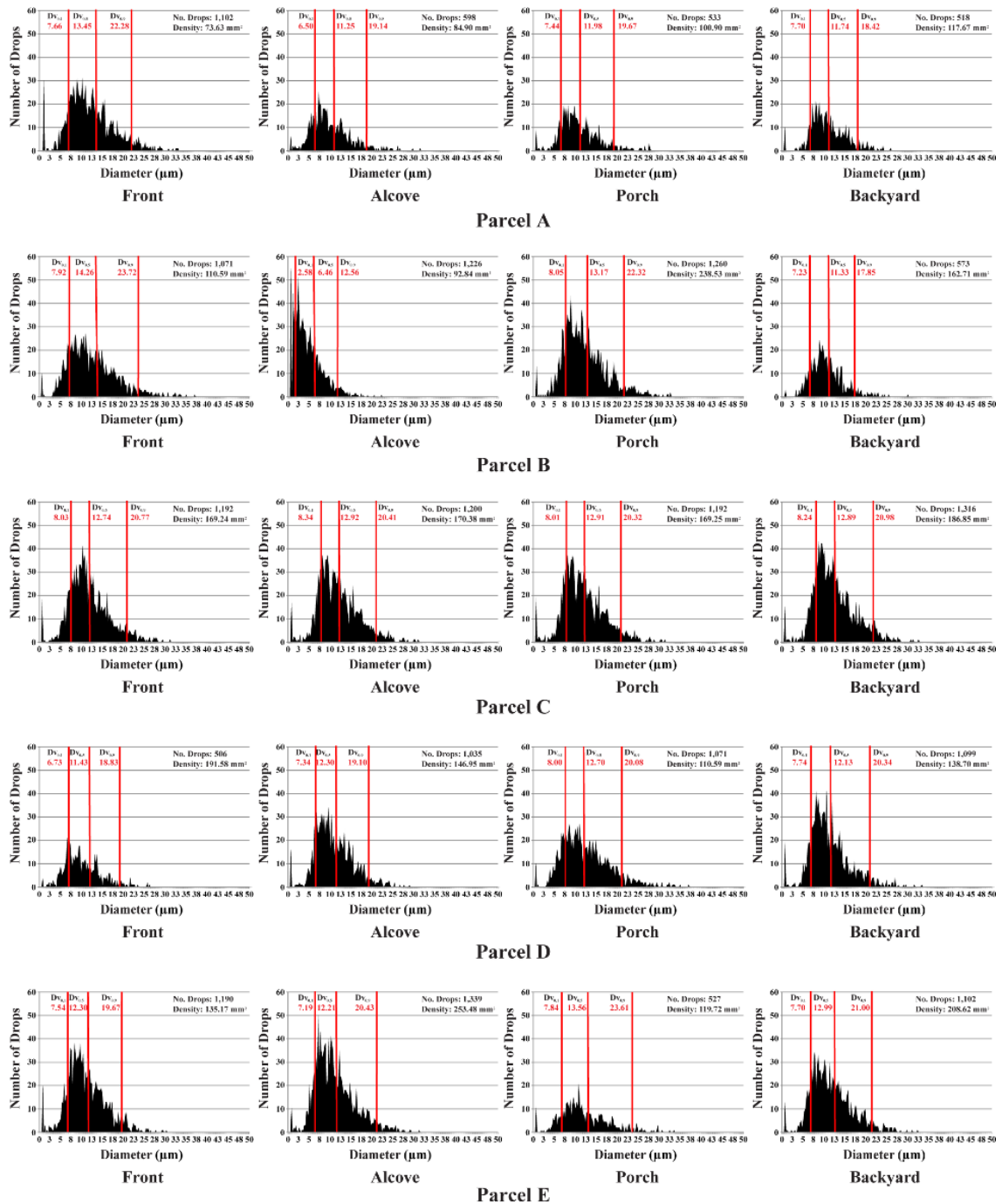


Figure I.3. Droplet characteristics of a max label ULV adjuvant application within individual stations and parcels in urban Mercer as sampled by FLB type impactors.

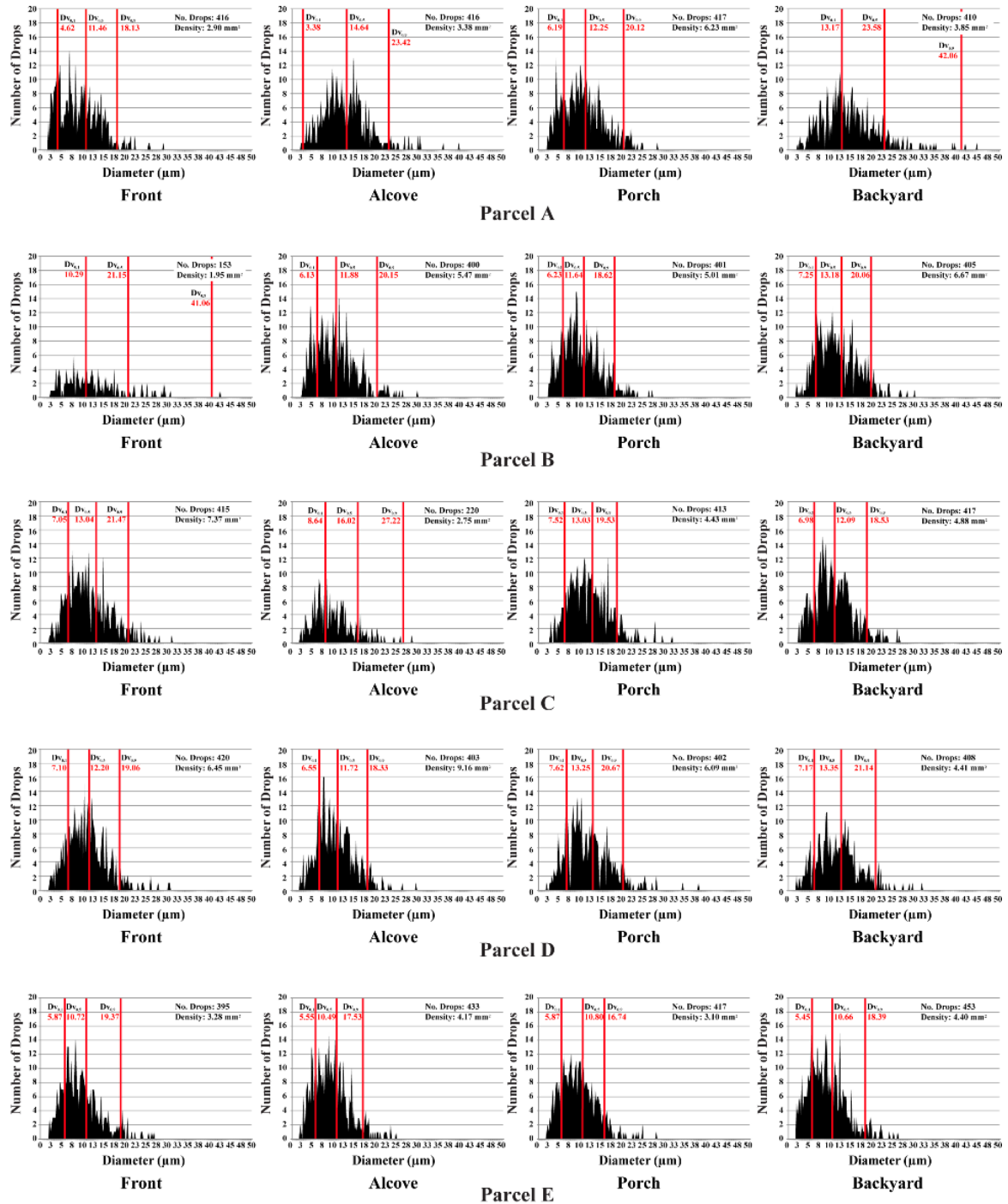


Figure I.4. Droplet characteristics of a mid label ULV adulticide application within individual stations and parcels in urban Mercer as sampled by Hock type impactors.

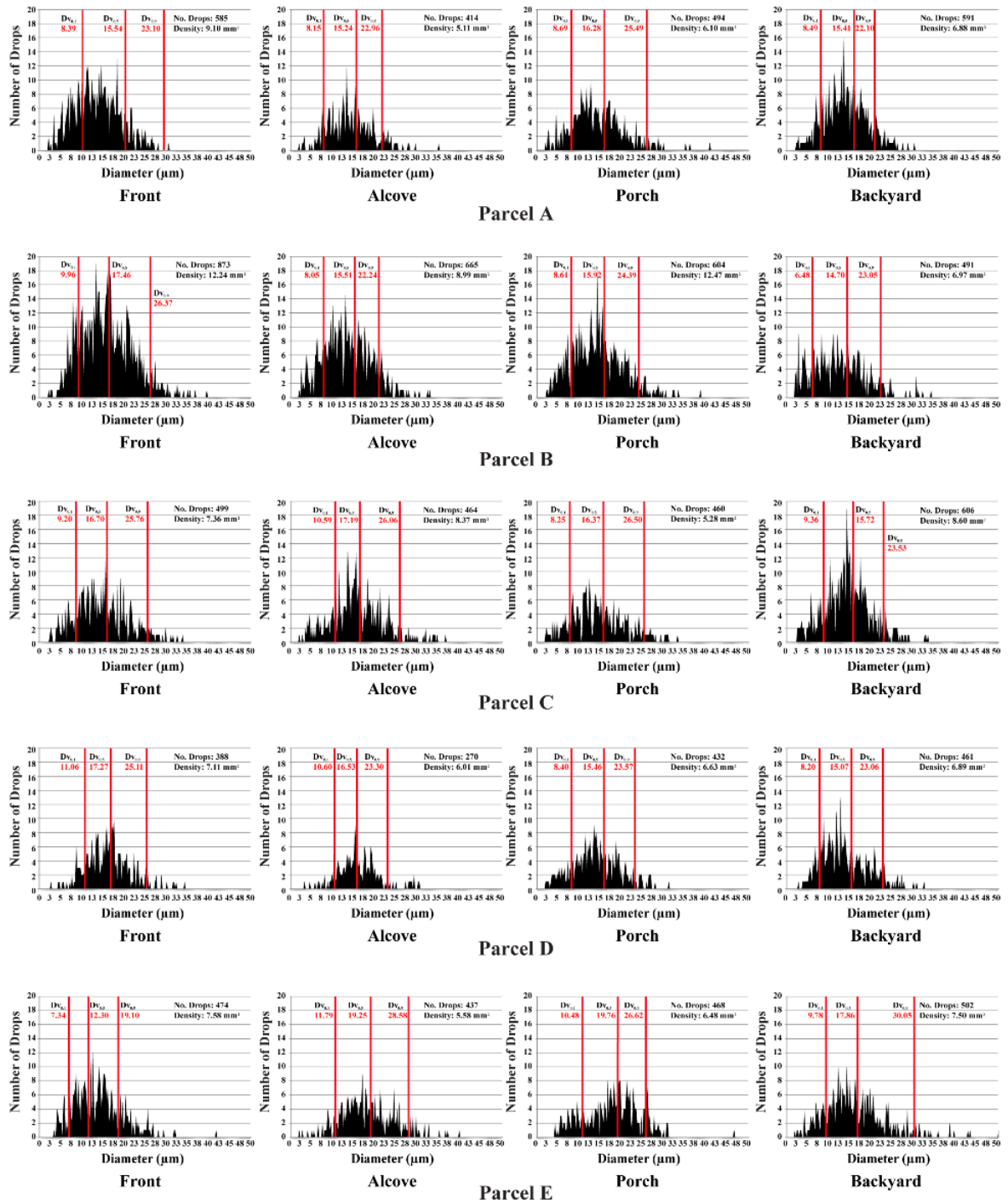


Figure I.5. Droplet characteristics of a max label ULV adjuvant application within individual stations and parcels in urban Mercer as sampled by Hock type impactors.

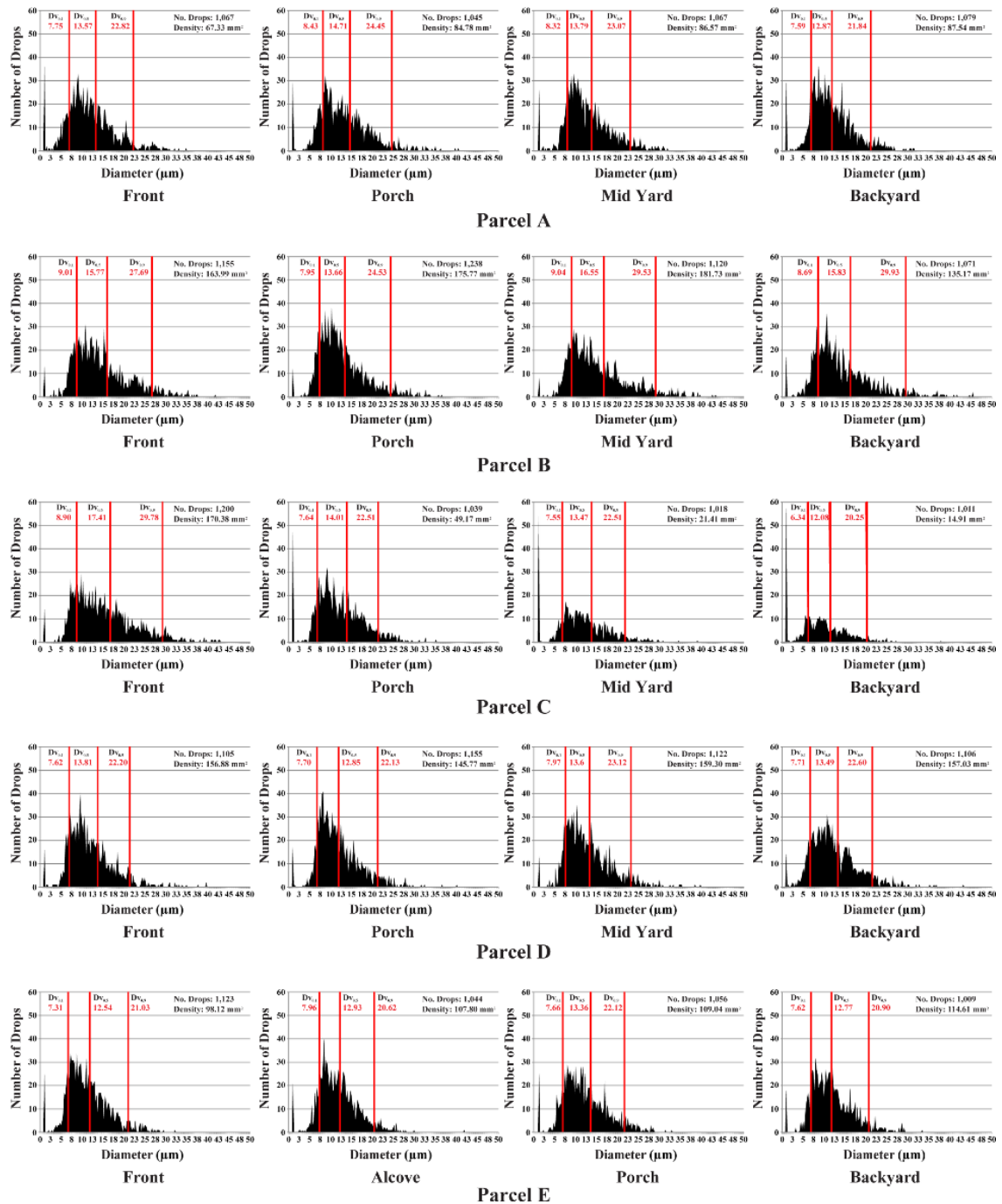


Figure I.6. Droplet characteristics of a max label ULV adulticide application within individual stations and parcels in suburban Monmouth, as sampled by FLB type impactors.

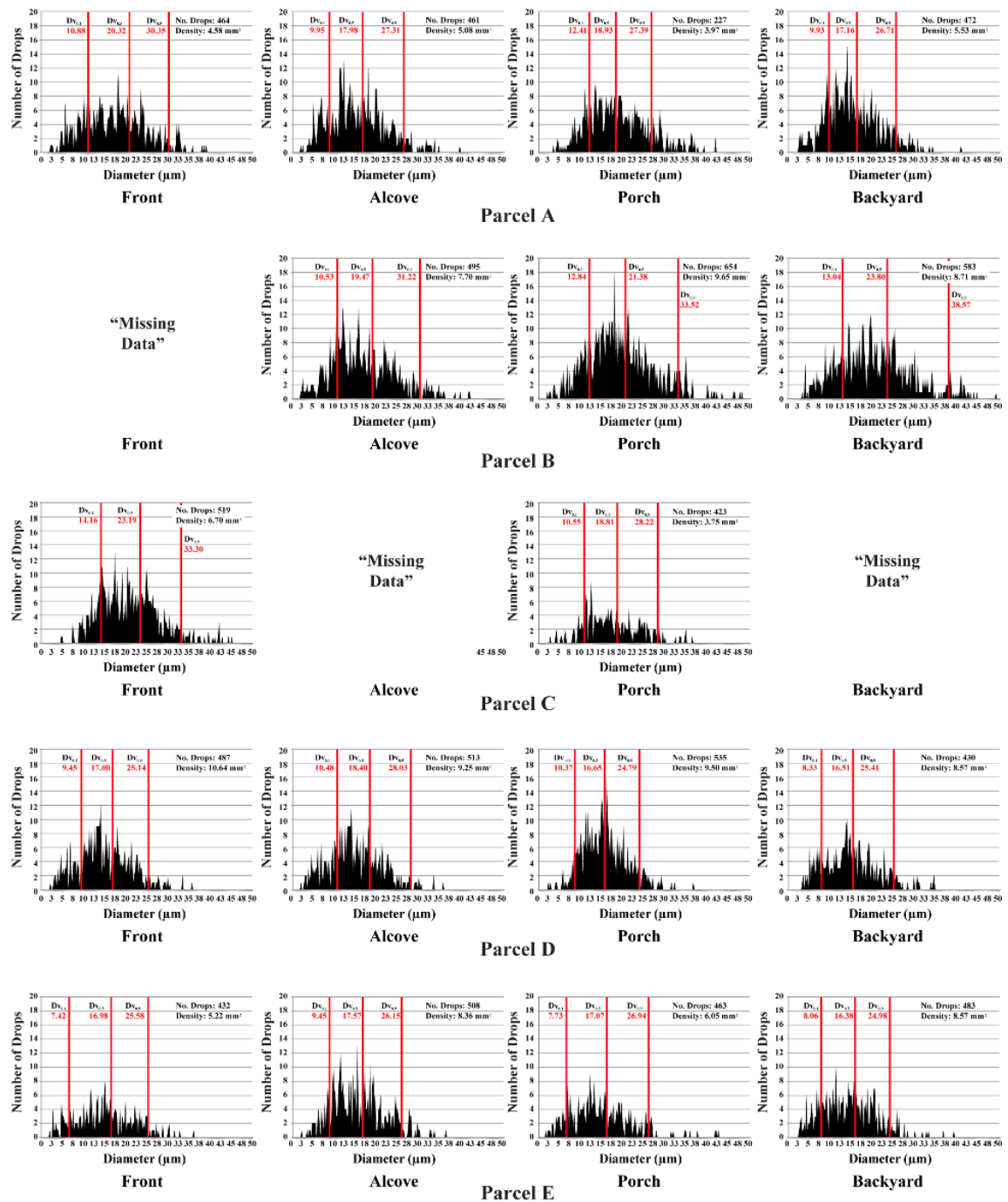


Figure I.7. Droplet characteristics of a max label ULV adulticide application within individual stations and parcels in suburban Monmouth, as sampled by Hock type impactors.

Appendix II

Additional Works on *Aedes albopictus* Biology and Control

The introduction and establishment of *Aedes albopictus* into the United States has had great significance on vector control programs tasked with protecting public health and comfort from mosquito species. Infestations of the Asian tiger mosquito present new challenges for public health programs already burdened with reduced economic budgets and available personnel. The species is a major biting nuisance that can affect human quality of life and socio-economics, in addition to being a competent vector of many arboviruses affecting human and veterinary health. *Aedes albopictus* will continue to expand its range, particularly into larger urban centers, and it will have a lingering impact on increasingly larger proportions of the human population. Only through increased knowledge of its biology, ecology, and effective control measures will public health practitioners be prepared to combat *Ae. albopictus*.

The overall theme that drove my research was the concern for public health and comfort and the opportunity to provide much needed information for the benefit of the greater vector control community. I have been fortunate to been involved as a co-principal investigator on the “Areawide Pest Management Program for the Asian Tiger Mosquito” (http://www.ars.usda.gov/research/projects/projects.htm?accn_no=412820). This program has elevated my education and given me the opportunity to provide significant and practical contributions to our respected field globally. In addition to countless presentations, workshops, and collaborations with numerous academic, federal, local, state, private, and public agencies, it has also provided me a direct opportunity to be involved in the development of a website on Asian tiger mosquitoes (<http://asiantigermosquito.rutgers.edu>) and the formation of numerous standard operating procedures describing various strategies optimized during the Areawide Project

(<http://asiantigermosquito.rutgers.edu/SOPsATM.html>). Additionally, my scholarly involvement has not been strictly bound to the main body of my dissertation. I have investigated various other aspects of the biology, ecology, and control measures relating to *Ae. albopictus*. Many of these investigations have culminated in peer-reviewed publications and many more are soon to follow. Some of the published body of work on *Ae. albopictus* that I have been directly involved in are provided below:

31. Unlu, I., **Faraji, A.**, Indelicato, N., and Fonseca, D. 2014. The hidden world of Asian tiger mosquitoes: immature *Aedes albopictus* (Diptera: Culicidae) dominate in rainwater corrugated extension spouts. Transactions of the Royal Society of Tropical Medicine and Hygiene. *Accepted*.
30. Williges, E., **Faraji, A.**, and Gaugler, R. 2014. Vertical oviposition preferences of *Aedes albopictus* in temperate North America. Journal of the American Mosquito Control Association. *Accepted*.
29. Marcombe, S., **Farajollahi, A.**, Healy, S., Clark, G., and Fonseca DM. 2014. Insecticide resistance status of United States populations of *Aedes albopictus* and mechanisms involved. PLoS One. *Accepted*.
28. Unlu, I., **Farajollahi, A.**, Rochlin, I., Crepeau, T., Healy, S.P., Fonseca, D.M., and Gaugler, R. 2014. Differences in male-female ratios of *Aedes albopictus* (Diptera: Culicidae) following ultra-low volume adulticide applications. Acta Tropica. *In press*.
27. Sun, D., Indelicato, N., Peterson, J., Williges, E., Unlu, I., and **Farajollahi, A.** 2014. Susceptibility of field-collected mosquitoes in central New Jersey to organophosphates and a pyrethroid. Journal of the American Mosquito Control Association. 30(2): 138-142.
26. Unlu, I. and **Farajollahi, A.** 2014. A multiyear surveillance for *Aedes albopictus* with Biogents Sentinel trap counts for males and species composition of other mosquito species. Journal of the American Mosquito Control Association. 30(2): 122-125.
25. Sun, D., Williges, E., Unlu, I., Healy, S., Crepeau, T., Williams, G., Obenauer, P., Hughes, T., Schoeler, G., Gaugler, R., Fonseca, D., and **Farajollahi, A.** 2014. Taming a tiger in the city: a comparison between motorized backpack applications and door-to-door source reduction efforts against *Aedes albopictus*. Journal of the American Mosquito Control Association. 30(2): 99-105.

24. Suman, D., **Farajollahi, A.**, Healy, S.P., Williams, G., Wang, Y., Schoeler, G., and Gaugler, R. 2014. Point source and area-wide field studies of pyriproxyfen autodissemination against urban container-inhabiting mosquitoes. *Acta Tropica*. 135: 96-103.
23. Halasa, Y., Shepard, D., Fonseca, D.M., **Farajollahi, A.**, Healy, S.P., Gaugler, R., Bartlett-Healy, K., Strickman, D., and Clark, G. 2014. Quantifying the impact of mosquitoes on quality of life and enjoyment of yard and porch activities in New Jersey. *PLoS One*. 9(3): e89221.
22. **Farajollahi, A.**, Williams, G., Condon, G.C., Kesavaraju, B., Unlu, I., Gaugler, R. 2013. Assessment of a direct application of two *Bacillus thuringiensis israelensis* formulations for immediate and residual control of *Aedes albopictus*. *Journal of the American Mosquito Control Association*. 29(4): 385-388.
21. Armstrong, P., Anderson, J., **Farajollahi, A.**, Healy, S.P., Unlu, I., Crepeau, T., Gaugler, R., Fonseca, D., and Andreadis, T. 2013. Isolations of Cache Valley virus from *Aedes albopictus* (Diptera: Culicidae) in New Jersey and evaluation of its role as a regional arbovirus vector. *Journal of Medical Entomology*. 50(6): 1310-1314.
20. Fonseca, D.M., Unlu, I., Crepeau, T., **Farajollahi, A.**, Healy, S.P., Bartlett-Healy, K., Strickman, D., Gaugler, R., Hamilton, G., Kline, D., and Clark, G.C. 2013. Area-wide management of *Aedes albopictus* II: gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes. *Pest Management Science*. 69: 1351-1361.
19. Unlu, I., **Farajollahi, A.**, Strickman, D., and Fonseca, D.M. 2013. Crouching tiger, hidden trouble: urban sources of *Aedes albopictus* (Diptera: Culicidae) refractory to source-reduction. *PLoS One*. 8(10): e77999.
18. **Farajollahi, A.** and Williams, G. 2013. An open-field efficacy trial using Aqua DUET™ via an ultra-low volume cold aerosol sprayer against caged Asian tiger mosquitoes, *Aedes albopictus*. *Journal of the American Mosquito Control Association*. 29(3): 304-308.
17. **Farajollahi, A.** and Price, D. 2013. A rapid identification guide for larvae of the most common North American container-inhabiting *Aedes* species of medical importance. *Journal of the American Mosquito Control Association*. 29(3): 203-221.
16. Crepeau, T., Unlu, I., Healy, S.P., **Farajollahi, A.**, and Fonseca, D.M. 2013. Experiences with the large scale operation of the BioGents Sentinel trap. *Journal of the American Mosquito Control Association*. 29(2): 177-180.

15. Rochlin, I., Gaugler, R., Williges, E., and **Farajollahi, A.** 2012. The rise of the invasives and decline of the natives: insights revealed from adult populations of container-inhabiting *Aedes* mosquitoes (Diptera: Culicidae) in temperate North America. *Biological Invasions*. 15(5): 991-1003.
14. Rochlin, I., Ninivaggi, D., Hutchinson, M., and **Farajollahi, A.** 2013. Climate change and range expansion of the Asian tiger mosquito (*Aedes albopictus*) in northeastern USA: implications for public health practitioners. *PLoS One*. 8(4): e60874.
13. Crepeau, T., Healy, S.P., Bartlett-Healy, K., Unlu, I., **Farajollahi, A.**, and Fonseca, D.M. 2013. Effects of BioGents Sentinel trap field placement on capture rates of adult Asian tiger mosquitoes, *Aedes albopictus*. *PLoS One*. 8(3): e60524.
12. Suman, D.S., Healy, S.P., **Farajollahi, A.**, Crans, S.C., and Gaugler, R. 2012. Efficacy of DUET™ dual-action adulticide against caged *Aedes albopictus* with the use of an ultra-low volume cold aerosol sprayer. *Journal of the American Mosquito Control Association*. 28(4): 338-340.
11. Unlu, I. and **Farajollahi, A.** 2012. To catch a tiger in a concrete jungle: operational challenges for trapping *Aedes albopictus* in an urban environment. *Journal of the American Mosquito Control Association*. 28(4): 334-337.
10. Halasa, Y.A., Shepard, D.S., Wittenberg, E., Fonseca, D.M., **Farajollahi, A.**, Healy, S., Gaugler, R., Strickman, D., and Clark, G.C. 2012. Willingness-to-pay for an area-wide integrated pest management program to control the Asian tiger mosquito in New Jersey. *Journal of the American Mosquito Control Association*. 28(3): 225-236.
9. Bartlett-Healy, K., Unlu, I., Obenauer, P., Hughes, T., Healy, S.P., Crepeau, T., **Farajollahi, A.**, Kesavaraju, B., Fonseca, D.M., Schoeler, G., Gaugler, R., and Strickman, D. 2012. Larval mosquito habitat utilization and community dynamics of *Aedes albopictus* and *Aedes japonicus japonicus* (Diptera: Culicidae). *Journal of Medical Entomology*. 49(4): 813-824.
8. Unlu, I. and **Farajollahi, A.** 2012. Vectors without borders: Imminent arrival, establishment, and public health implications of the Asian bush (*Aedes japonicus*) and Asian tiger (*Aedes albopictus*) mosquitoes in Turkey. *Hacettepe Journal of Biology and Chemistry*. 40(1): 23-36.
7. Bartlett-Healy, K., Hamilton, G., Healy, S.P., Crepeau, T., Unlu, I., **Farajollahi, A.**, Fonseca, D.M., Gaugler, R., Clark, G.C., and Strickman, D. 2011. Source reduction behavior as an independent measurement of the impact of a public health education campaign in an integrated vector management program for the Asian tiger mosquito. *International Journal of Environmental Research and Public Health*. 8(5): 1358-1367.

6. Unlu, I., **Farajollahi, A.**, Healy, S.P., Crepeau, T., Bartlett-Healy, K., Williges, E., Strickman, D., Clark, G.C., Gaugler, R., and Fonseca, D.M. 2010. Area-wide management of the Asian tiger mosquito: Choice of study sites based on geospatial characteristics, socioeconomic factors, and mosquito populations. *Pest Management Science*. 67(8): 965-974.
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3. Nelder, M.P., Kesavaraju, B., **Farajollahi, A.**, Healy, S.P., Unlu, I., Crepeau, T., Raghavendran, A., Fonseca, D.M., and Gaugler, R. 2010. Suppressing *Aedes albopictus*, an emerging vector of dengue and chikungunya viruses, by a novel application of a monomolecular film and an insect growth regulator. *American Journal of Tropical Medicine and Hygiene*. 82(5): 831-837.
2. **Farajollahi, A.** and Nelder, M.P. 2009. Changes in *Aedes albopictus* (Diptera: Culicidae) populations in New Jersey and implications for arbovirus transmission. *Journal of Medical Entomology*. 46(5): 1220-1224.
1. **Farajollahi, A.**, Kesavaraju, B., Price, D.C., Williams, G.M., Healy, S.P., Gaugler, R., and Nelder, M.P. 2009. Field efficacy of BG-sentinel™ and industry-standard traps for *Aedes albopictus* (Diptera: Culicidae) and West Nile virus surveillance. *Journal of Medical Entomology*. 46(4): 919-925.

Acknowledgment of Previous Publications

Citations for Chapters 1-3 are as follows:

Faraji, A., A. Egizi, D. M. Fonseca, I. Unlu, T. Crepeau, S. P. Healy, and R. Gaugler.

2014. Comparative host feeding patterns of the Asian tiger mosquito, *Aedes albopictus*, in urban and suburban northeastern USA and implications for disease transmission. PLoS Neglected Tropical Diseases. *In press*.

Faraji, A., I. Unlu, T. Crepeau, S. P. Healy, S. P. Crans, G. Lizarraga, D. M. Fonseca, and

R. Gaugler. 2014. Droplet penetration and characterization of an ultra-low volume mosquito adulticide spray within urban and suburban environments of northeastern USA. Pest Management Science. *Submitted*.

Farajollahi, A., S. P. Healy, I. Unlu, R. Gaugler, and D. M. Fonseca. 2012. Effectiveness

of ultra-low volume nighttime applications of an adulticide against diurnal *Aedes albopictus*, a critical vector of dengue and chikungunya viruses. PLoS One.

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