TECHNICAL BULLETIN OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION

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TECHNICAL BULLETIN OF THE FLORIDA MOSQUITO CONTROL ASSOCIATION

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ARBOVIRUS SURVEILLANCE
AND MOSQUITO CONTROL WORKSHOP

A volume of selected papers from:
The 11th workshop, March 25-27, 2014, and the 12th workshop, March 24-26, 2015, and Anastasia Mosquito Control District and its Collaborating Organizations

Edited by:
Rui-De Xue

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INTRODUCTION

During 2014 and 2015 the Anastasia Mosquito Control District (AMCD) of St. Johns County, Florida held its eleventh and twelfth annual Arbovirus Surveillance and Mosquito Control Workshops at District headquarters in St. Augustine, Florida. Both workshops were jointly sponsored by the AMCD and the USDA, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, FL. These workshops were designed to facilitate the exchange of information regarding mosquito-borne diseases, review recent research and developments in arbovirus and mosquito surveillance, mosquito control, and to offer unique training opportunities for mosquito control professionals.

The eleventh workshop was held from March 25-27, 2014, and included 66 presentations divided into 9 sessions. Due to the outbreak of eastern equine encephalitis virus (EEE) in the USA in 2013, the prevention and control of this disease was the theme for this workshop. Thomas Unnasch from University of South Florida, Tampa, was the keynote speaker and presented some new insights into the ecology of eastern equine encephalitis virus transmission in the southeastern United States. Scientists from Mali, Malaysia, Saudi Arabia, China, Brazil, and Israel, USDA/CMAVE, Navy Entomology Center of Excellence, universities, as well as mosquito control professionals from industry, state and local governmental agencies provided presentations that included updates on mosquito surveillance and control and review of new mosquito control products and equipment.

The twelfth workshop was held from March 24-26, 2015, and included 73 presentations divided into 7 sessions. Due to the outbreak of chikungunya in the Caribbean basin in 2014, the prevention and control of this disease was the theme for this workshop. John Beier from University of Miami Miller School of Medicine was the keynote speaker and provided information on the chikungunya outbreak and transmission risk assessments. Randy Gaugler from Rutgers University, New Jersey was the domestic guest speaker and presented a summary of activities from the Center for Vector Biology located on the main campus. Scott Ritchie, from Australia, gave the international guest speaker presentation on his work with honey cards for arbovirus detection. Scientists from USDA/CMAVE, Navy Entomology Center of Excellence, universities, as well as mosquito control professionals from industry, state and local governmental agencies all provided presentations that included the latest updates on mosquito surveillance and control methods as well as control products and equipment.

A total of 40 continuing education credits (CEU) were provided to workshop attendees between 2014 and 2015. We are especially appreciative of the speakers and contributors who gave presentations in these workshops and to those who submitted manuscripts for this volume, as well as the organizations/companies who provided partial funding for the workshops. We thank those who reviewed manuscripts prior to publication, including Lisa Drake*, Whitney Qualls*, Mohamed Sallam*, Ali Fulcher*, Jennifer Gibson*, Michael L. Smith, John C. Beier*, Barry Tyler, Larry Hribar*, Peter Jiang*, Gunter Muller*, Edith Revery, James Cilek, Sandy Allan, Seth Britch, Muhammad Farooq, Phil Kaufman, Tianyun (Steven) Su, Andrew Li, Aaron Lloyd, Gregg Ross, Richard Weaver, Donald Barnard, Dan Kline, Jerry Hogsette, Jodi Scott*, Christopher Bibbs*, and Charolette Hall* (* indicates reviewers who reviewed more than one manuscript). We also acknowledge the support and encouragement of the AMCD Board of Commissioners, administrative office, District staff, and industry.

Rui-De Xue, Ph.D.
Director, Anastasia Mosquito Control District
**ABSTRACT.** *Aedes albopictus* is a vector of dengue and chikungunya viruses. In recent years, this species has expanded its range to the Americas, Europe, and Africa. Host seeking and blood feeding is one of the most important behaviors mosquitoes possess. Understanding both behaviors in *Ae. albopictus* may result in more effective control of this species from a nuisance standpoint, as well as pathogen transmission. The topics discussed in this review include: olfaction, adult ec dysis (as it relates to first host seeking attempt), frequency of host seeking and blood feeding, biting cycle and blood feeding pattern, feeding location and biting behavior, host preference, multiple blood feeding behavior, blood meal size, artificial blood feeding, and a few other miscellaneous factors that have been reported to influence host seeking and blood feeding behavior in this species.

**Key Words:** host seeking, blood feeding, blood meal, *Aedes albopictus*

**I. INTRODUCTION**

The Asian tiger mosquito, *Aedes albopictus* (Skuse), is an important vector of dengue and chikungunya viruses and a competent vector of several other arboviruses including yellow fever, Ross River virus, West Nile virus, La Crosse virus, and eastern equine encephalitis virus (Mitchell 1991, Ali & Nayar 1997, Gratz 2004). A considerable body of work has arisen since its invasion into the Americas and Europe in the middle of the 1980’s (Hawley 1988, Francy et al. 1990, Rai 1991, Bonizzoni et al. 2013). Host seeking and blood feeding in *Ae. albopictus* is a major part of female mosquito behavior and a requirement for reproduction. This critical behavior is directly related to transmission of pathogens and has driven the pathway of research to identify and develop attractants and repellents for prevention and control of mosquitoes. This paper reviews some of the progress made on *Ae. albopictus* behavior as it relates to finding and successfully feeding on a suitable host.

**II. OLFATORY STRUCTURES**

Visual, thermal, and olfactory stimuli all contribute to host seeking and blood feeding behavior in female mosquitoes (Bowen 1991). The internal, external ultrastructure (as well as possible biological features) of virtually all olfactory sensilla on the antennae of *Ae. aegypti* (L.) and *Anopheles stephensi* Liston have been summarized in a review by Sutcliffe (1994). He found that small and large sensilla coeloconica, ampullaceae, grooved pegs, and trichodea existed on mosquito antennae, as well as capitate pegs on mosquito palps. Sensilla coeloconica and ampullaceae may respond to humidity and temperature while trichodea appear to have a sensory function that may distinguish oviposition site-related compounds, essential oils, or fatty acids associated with human skin and certain repellents (Sutcliffe 1994). Grooved pegs were also reported to have an olfactory function that responded to ammonia, acetone, acetic acid, anisole, and lactic acid. Capitate pegs on mosquito palpi responded to carbon dioxide (Sutcliffe 1994). Subsequently, the type, distribution, and fine structure of sensillae on the surface of the antenna of *Ae. albopictus* was studied using scanning and transmission electron microscope methods by Huang et al. (1991), Xue et al. (1991), and Seenivasagan et al. (2009). One of the olfactory structures on the antenna of Asian tiger mosquitoes was
sensilla trichoidea that possessed a pore at their tip with many pores through the hair wall. Other structures such as trichodea were numerously distributed along all flagella segments of the antenna and consisted of long or short hairs with sharp or blunt tips. In addition, long and short types of grooved peg sensillae were also observed on the antenna. Sensilla coeloconicae were observed in the terminal flagellum of *Ae. albopictus* while sensilla chaeticae were distributed throughout the body surface and revealed greater variation in morphology and morphometric parameters (Seenivasagan et al. 2009). The four types of sensillae from antenna of *Ae. albopictus* are chaetica (19%), trichodea (71%), grooved pegs (9%), and coeloconica (1%), based on their morphological characters, thickness of hair wall, pore density, and dendrite number, respectively. Interestingly, Huang et al. (1991) found that olfactory sensillae, in mosquitoes, may be stimulated by host odors before any host seeking behavior occurs.

**III. ADULT ECDYSIS TO FIRST HOST SEEKING**

After adult emergence, juvenile hormone secretion plays an important role in the physiological regulatory process in most female mosquitoes before host seeking and blood feeding (Huang and Xue 1991, Clements 1999, Meola and Readio 1988, Klowden 1990, Hansen et al. 2014). However, in *Ae. aegypti*, host-seeking was either independent of juvenile hormone concentration or the sensitivity threshold was very different than that of the ovaries (Bowen 1991) a process that may be similar for *Ae. albopictus*.

Several workers have observed that the time from adult *Ae. albopictus* emergence to the beginning of host seeking and blood feeding varied, depending on mosquito strain. del Rosario (1963), Gubler and Bhattacharya (1971), Hien (1976), and Huang and Gao (1991) reported that this period was about 2-3 days, at 24-29°C, for strains from Calcutta, Vietnam, Philippines, and Shanghai. The median time from emergence to host seeking and blood feeding at 25°C was 2.5 days for an *Ae. albopictus* population from Nagasaki, Japan, but this time increased to 3.5-4.5 days when adults were produced by rearing larvae at high densities (Mori 1979).

**IV. FREQUENCY OF HOST SEEKING AND BLOOD FEEDING**

Host seeking activity of *Ae. albopictus*, as with any other species, is stimulated by certain chemical odors and compounds (Huang et al. 1991b, Hao et al. 2008, Guha et al. 2014). Klowden and Briegel (1994) reported that host seeking was inhibited during the gonotrophic cycle (as measured with an olfactometer). Furthermore, Fukumitsu et al. (2012) reported that the concentration of a neurotransmitter, dopamine, declined in the head of *Ae. albopictus* as host-seeking increased.

The behavioral sequence of host seeking and blood feeding in *Ae. albopictus* is similar to other species of mosquitoes; these steps include, orientation, landing, probing, and feeding as described by Hocking (1971), Edman and Spielman (1988), Bowen (1991), and Takken (1991). Also, the frequency of host seeking and blood feeding depends largely upon longevity and number of gonotrophic cycles. Generally, female *Ae. albopictus* live from 4 to 8 weeks in the laboratory at 25°C but may survive up to 3-6 months outdoors (Hawley 1988, Xue et al. 2010). A female can take about 1 week to complete a gonotrophic cycle (the interval between successive oviposition) (Hawley 1988) and may complete 4 to 8 gonotrophic cycles during her lifetime, according to a laboratory study by Fu et al. (1982). In order to complete these numbers of gonotrophic cycles, *Ae. albopictus* females would need to seek out hosts and feed at least 4 to 8 times (Fu et al. 1982). However, this may not be absolute, because under laboratory conditions, a Shenzhen strain of *Ae. albopictus* fed on a human subject twice during one gonotrophic cycle and 10 times during 7 gonotrophic cycles (Liu Yang unpubl data). Field data from Liu (1965) showed that the females, in their study, had usually completed 1-2 gonotrophic cycles in their lifetime, according to the
number of observable follicular dilations. But interpretation of field data must be approached with caution as the number of follicular dilations is often fewer than the actual number of gonotrophic cycles completed. For example, no more than 3 dilations were observed from field collected *Ae. albopictus* even though those individuals were known to have completed 4 or 5 gonotrophic cycles (Liu, 1965). Moreover, if longevity of this mosquito species is based on parity status (i.e. gonotrophic cycles) the data may underestimate age (Mori and Wada 1977).

Time between oviposition events can vary with geographic strains of *Ae. albopictus*. Gubler and Bhattacharya (1971) reported that a Vietnam strain of this mosquito species oviposited every 4.6 days at 26° C and from 3-5 days at 25° C for a Shanghai and Guangxi strain from China (Li, 1991). These latter authors also noted that oviposition interval time was protracted at 20º C to at least 10 days and considerably shortened to 4.5-6 days at 30° C.

The numbers of blood meals, and frequency of host seeking, were negatively correlated with body size in *Ae. albopictus* (Hawley 1988, Farjana and Tuno 2013). Moreover, several authors subsequently observed that frequency of host seeking and blood feeding, during a gonotrophic cycle, depended on a variety of factors that included, mosquito age (Xue et al. 1995), body size (Xue & Barnard 1996, 2012, Farjana and Tuno 2013), blood meal size and mating status (Klwooden, 1988, Lee and Klowden 1999, Barnard and Xue 2009), parity and time of day (Xue and Barnard 1996), carbohydrate availability and fatigue (Xue and Barnard 1999, Xue and Debboun 2014), host availability, species of host and protective behavior, spatial repellents (Hao et al. 2008, 2013), and other miscellaneous factors (Xue et al. 1995, Xue and Debboun 2014).

### V. BITING CYCLE AND BLOOD FEEDING PATTERN

Kawada and Takagi (2004) used a photoelectric sensor to record the nocturnal behavior of *Ae. albopictus* and found that host seeking was positively correlated with increasing light intensity where the threshold intensity was >10 lux (approximately 1 foot candle) (Kawada et al. 2005). However, these authors added that complete darkness during the daytime deactivated host seeking behavior. Earlier, Hawley (1988) stated that Asian tiger mosquitoes primarily sought hosts and blood fed during the day and rarely at night. Costanaza et al. (2015) also found that host seeking activity varied with light levels (0 to 440 lux). Consistent biting activity in their laboratory studies occurred at a photoperiod of 12L:12D.

Kawada and Takagi (2004) further observed that there were two peaks of diel activity in female *Ae. albopictus* one at 1000 to 1200 and another from 1400 to 1600 h. Under complete darkness, biting activity did not peak, but under a constant light regime, biting peaked at 2200-2300 (Huang and Gao 1991a, Xue 1991). The USDA Gainesville, Florida strain, reared under different photoperiod regimes in the laboratory, did not show temporal significant differences in blood feeding frequency. Also short photoperiods did not affect blood-feeding activity of this strain (Table 1). Fu (1990) also reported that outdoor biting activity peaked in early morning (7-9 am) and late afternoon (5-8 pm) in strains from Shanghai.

### Table 1. Effects of photoperiod (at 25° C) on *Aedes albopictus* first gonotrophic feeding success using baby chickens under laboratory conditions (Xue unpubl data)*

<table>
<thead>
<tr>
<th>Photoperiod regime (h)</th>
<th>No. tested</th>
<th>Blood fed (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L:D=LL</td>
<td>195</td>
<td>74.9 ± 6.2</td>
</tr>
<tr>
<td>L:D=14:10</td>
<td>400</td>
<td>66.8 ± 2.0</td>
</tr>
<tr>
<td>L:D=12:12</td>
<td>240</td>
<td>64.2 ± 4.0</td>
</tr>
<tr>
<td>L:D=08:16</td>
<td>240</td>
<td>59.2 ± 4.0</td>
</tr>
<tr>
<td>L:D=DD</td>
<td>240</td>
<td>65.5 ± 7.6</td>
</tr>
</tbody>
</table>

*1Sugar water (3%) was provided when rearing adults. Six day old mosquitoes used in study, 1 hour blood feeding time.
2Photoperiod regime of fourth instar larvae in rearing chamber: L=light, D=dark, LL=continuous light, DD=continuous darkness.
Fujian, Jiangsu, Guangxi and Henan, China. A study by Delatte et al. (2010) also found that Ae. albopictus exhibited a bimodal daily feeding activity in La Reunion where 89% of the mosquito population was exophagic and 87% were exophilic.

Peak biting activity of Ae. albopictus also appears to vary with location and habitat. A clear bimodal pattern (early morning and late afternoon) of feeding activity has been observed in Asian countries, such as China, Japan, Philippines, and Singapore (Ho et al. 1973). In southern Brazil, biting activity occurred during early morning, early evening, and late night with peaks at 6 am and 10 pm but the greatest activity was between 4-5pm (Margues & Gomes 1997). Interestingly, sugar feeding and host seeking rhythm in Ae. albopictus, recorded in the laboratory using remote sensors, discovered that the evening sugar feeding period was similar to the evening host seeking period (Yee and Foster 1992).

In the laboratory, an indoor population showed only a single broad period of activity in the afternoon (Wang, 1962). However, the biting activities of a laboratory Shanghai strain of Ae. albopictus showed that females would feed on a host anytime during 24 hours regardless of long or short photoperiods (Huang & Gao 1991a). Also, the USDA Gainesville laboratory strain of this species will also seek, bite, and feed on human hosts anytime during a 24 hour period but has early morning and late afternoon biting peaks (Xue and Barnard 1996, 1997).

In summary, laboratory and field studies have shown, without a doubt, that most strains of Ae. albopictus are bimodal daytime biters with a small peak in the early morning and a larger peak in the late afternoon. Those strains that do not show this bimodality are probably highly inbred laboratory colonies reared under artificial light regime conditions.

VI. FEEDING LOCATION AND BITING BEHAVIOR

Female Ae. albopictus prefer to bite exposed human skin primarily around the ankles and knees (Robertson and Hu 1935). For chickens, this mosquito species will usually bite the head, feet, and other exposed skin, according to this author’s observations (Xue unpubl). Although Ae. albopictus are usually outdoor biting species, biting females can be collected from indoors.

Most female Ae. albopictus take 1-2 minutes to completed engorgement. Some strains required 3.6 minutes to engorge on a human arm (Soekiman et al. 1984 in Hawley 1988). Fu (1990) found this species took 20-30 seconds from probing to abdomen distention at 23-33°C and RH 68-95%. The complete feeding period from probing to engorgement averaged 104.14 seconds (range 70-160 seconds). The temperature and humidity change during experiments did not significantly influence feeding persistence (Fu, 1990). Hien (1976) also found that biting persistence of Ae. albopictus varied according to strain, age, body size, host behavior, and environmental conditions.

VII. HOST PREFERENCE

Host preference in Ae. albopictus can directly affect vector competence and transmission risk of mosquito-borne disease pathogens. Determination of host preference by mosquito vectors is usually obtained by a variety of animal baits or traps (Takken and Verhulst 2013). Blood meal source identification in Asian tiger mosquitoes can be obtained by a variety of laboratory methods, such as sera precipitin (Tempelis 1975) agar gel-precipitin tests (Sivan et al. 2015), direct or indirect enzyme-linked immunosorbent assays (ELISA) using antisera (Ponlawat and Harrington, 2005), and polymerase chain reaction (PCR) (Richards et al 2006, Egizi et al 2013).

In the laboratory, there are two methods that can be used to determine host preference of female Ae. albopictus. Olfactometers are often used to test host odors from different animals. Using this method, Miyagi (1972) and Gubler (1970) determined that Ae. albopictus preferred feeding on mammals, including mice/rats, guinea pigs, dogs, cows, and humans. These authors also found that chickens, snakes, turtles, and frogs can be fed upon by this mosquito species (Miyagi
A second method employed by Hess et al. (1968) used forage ratios for determination of host preference obtained by precipitin tests. His results showed a mammal forage ratio >10 times that of birds, but female *Ae. albopictus* could feed on birds when mammals were unavailable. Konishi’s (1989) laboratory experimental results showed that *Ae. albopictus* preferred to feed on humans compared with canines when exposed concurrently. However, the authors acknowledge that feeding pattern in the laboratory and field were influenced primarily by host availability.

In Japan and rural Singapore (Kim et al. 2009, Sawabe et al. 2010, Kek et al. 2014) *Ae. albopictus* reportedly fed on mammals. In the USA, this species preferred mammals and birds (Passeriformes, Columbiformes, and Ciconiiformes) as well as rats, dogs, humans, rabbits, deer, squirrels, opossums, raccoons, bovine, (Savage et al. 1993, Niebylski et al. 1994, Faraji et al. 2014, Valerio et al. 2010, 2010a). Asian tiger mosquitoes in Hawaii, also preferred to feed on mammals, including dogs, cows, humans, cats, monegese, pigs, and horses (Tempelis 1975, Tempe- litis et al. 1970). In Missouri and New Jersey Savage et al. (1993) and Faraji et al. (2014), respectively, found only a small percentage of blood meals from birds in their field collections. Sullivan et al. (1971) reported that *Ae. albopictus* commonly fed on a variety of hosts including humans, buffalo, pigs, dogs, and chickens on the island of Koh Samui, Thailand with an even higher percentage of human feeding detected by sandwich-B enzyme-linked immunosorbent assays (Ponlawat & Harrington 2005). These authors ranked the blood feeding pattern of *Ae. albopictus* (top 4 host species) as follows: (1) humans, (2) bovine, (3) swine, and (4) cat, rat, chicken (equal numbers detected). They concluded that nonhuman hosts provided a significant source of the blood meals for *Ae. albopictus* in Thailand. However, precipitin tests, ELISA, and PCR were used to investigate host-feeding patterns of 172 blood-fed Asian tiger mosquitoes collected from Potosi, Missouri, during the summers of 1989-1990. In that study, no blood meals from cats, horses, rats, or swine were detected in those mosquitoes.

The majority of blood meals (83.2%) from *Ae. albopictus* in rural and semi-urban areas of Singapore were from humans with a small percentage from shrews, swine, dogs, cats, turtles, and multiple hosts in rural settings (Kek et al. 2014). However, in that same study blood meals from Asian tiger mosquitoes in the urban areas were entirely from humans. Faraji et al. (2014) reported that *Ae. albopictus* from Mercer County, New Jersey fed on mammalian hosts with over 90% of their blood meals derived from humans (58%) and domestic pets (23% cats and 15% dogs). This mosquito species fed on humans significantly more in suburban than in urban areas and cat-derived blood meals were greater in urban habitats while were no avian-derived blood meals were detected. In a central North Carolina suburban landscape study, 7% of blood meals from *Ae. albopictus* were avian hosts while 83% were mammalian that included humans (24%), cats (21%), and dogs (14%) (Richards et al. 2006). Blood from Asian tiger mosquitoes was exclusively human (100%) in urban zones from Spain and southern Thailand and 95% from Cameroon (Munnoz et al. 2011, Ponlawat and Harrington 2005, Kamgang et al. 2012, respectively). The results show that the *Ae. albopictus* populations in the New World are opportunistic feeders that use a variety of hosts from cold-blooded to warm-blooded animals (Delatte et al. 2010). This fact has the potential for this mosquito species to become involved in the transmission cycle of indigenous arboviruses (Savage et al. 1993). Conversely, feeding preference may also limit the vector potential of *Ae. albopictus* for some arboviruses but the high mammalian affinity of this mosquito species suggests that it can be an efficient vector of mammal-and human-driven zoonoses such as dengue, La Crosse, and chikungunya viruses.

**VIII. MULTIPLE BLOOD-FEEDING BEHAVIOR**

*Aedes albopictus* egg development is initiated by the blood meal (Xue et al. 2009).
This developmental process is linked to the type of host, frequency, and volume of blood that a mosquito can obtain. It is known that anti-mosquito behavior by a host can adversely influence the amount of blood that mosquitoes will have available to mature eggs (Walker and Edman, 1985). Other factors related to the mosquito itself (i.e. body size, age, and pathogen infection), may result in reduced fecundity or failure of egg maturation (Klowden 1988, Xue and Edman 1991). For example, female *Ae. albopictus* blood fed on guinea pig and human hosts produced significantly more eggs compared with other species. Fecundity in mosquitoes that took a double blood meal (chicken and guinea pig), a triple blood meal (3 separate guinea pigs), or mixed blood meals (chickens, guinea pigs, and humans) produced significantly more eggs than mosquitoes fed once or twice on a single chicken. Also, triple-feeding or mixed feeding (3 meals on 3 host types) decreased the gonotrophic dissociation frequency in those mosquitoes (Xue et al. 2008, 2009). Gonotrophic dissociation occurs when eggs fail to mature after a blood meal has been obtained [Xue et al. 2009].

Boreham (1976) suggested that multiple blood-feeding in vector mosquitoes increased the probability of pathogen transmission. Because *Ae. albopictus* will engage in multiple blood-feeding its’ risk of pathogen infection and transmission is greater than species that do not possess this ability. Indeed, Shroyer (1986), Lu (1990), Mitchell (1991), Rai (1991), and Gratz (2004) have found that Asian strains of this mosquito species possessed a high susceptibility to several arboviruses, including dengue, in the laboratory. Also, Xue and Barnard (1996) found that multiple feeding and host-seeking behaviors were negatively correlated with body size. Small body size tended to have more contact with a host due to low nutrition (Xue and Barnard 1996).

In the laboratory, *Ae. albopictus* must feed on blood at least once for egg maturation. About 12.5% of a strain from Vietnam required two blood meals to successfully oviposit their first batch of eggs. Gubler and Bhattacharya (1971) found that females in the later gonotrophic cycles required two blood meals, but never exceeded 22% of the population. Mori (1979) reported the number of blood meals required was dependent on mosquito strain, nutrition, host behavior, and environmental conditions. Klowden and Briegel (1994) also observed that if *Ae. albopictus* was allowed to blood-feed to repletion, its host-seeking behavior was inhibited during that gonotrophic cycle.

In laboratory studies, Fu (1982, 1990) found that about 13-50% of engorged *Ae. albopictus* were able to take a second blood meal from a mouse or human arm (Table 2). Furthermore, when Fu (1990) dissected ovaries of females that had taken a second blood meal 60, 75, and 100 hours after their first blood meal they had produced about 98 to 102 eggs/female while 84.4% of those individuals had already laid some eggs. In the field, 5% gravid females were collected from human legs in biting collections whereas 37.8% gravid females were collected from

<table>
<thead>
<tr>
<th>Time after initial blood meal (h)</th>
<th>Mice</th>
<th>Human arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>198</td>
<td>10.0</td>
</tr>
<tr>
<td>24</td>
<td>199</td>
<td>9.5</td>
</tr>
<tr>
<td>36</td>
<td>200</td>
<td>8.5</td>
</tr>
<tr>
<td>48</td>
<td>199</td>
<td>6.0</td>
</tr>
<tr>
<td>60</td>
<td>200</td>
<td>17.5</td>
</tr>
<tr>
<td>72</td>
<td>100</td>
<td>4.0</td>
</tr>
</tbody>
</table>

aNA=not available

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Table 2. Mean percentage of secondary blood meals from a *Aedes albopictus* Guangzhou, China strain under laboratory conditions (from Fu 1990).
human hosts indoors (Fu, 1982). In that field study, 8.3% of collections contained gravid females that had obtained a second blood meal during the 24 h observation period. In that same study, second blood meals by *Ae. albopictus* were observed in gravid individuals at each hourly sample interval during biting cycles.

Other field studies where multiple blood feeding by *Ae. albopictus* has been reported involve mixed blood meals from avian and non-avian hosts (Richards et al. 2006) or humans and other mammals (Delatte et al. 2010). Ponlawat and Harrington (2005) found that 3.8% of *Ae. albopictus* populations contained a mixture of swine-human blood while <1% of blood meals were dog-human or cat-human. Multiple host blood meals have also been reported from this mosquito species collected from rural settings in Singapore (Kek et al. 2014). Also, Tempelis et al. (1970) reported 0.3% mixed blood meals in an *Ae. albopictus* population from Hawaii. Molecular analysis (cytochrome b) of blood meals from individuals of this species collected from 2 South Carolina zoological parks revealed that the mosquitoes readily fed on the captive animals, as well as the local humans and wildlife, resulting in mixed blood meals (Tuten et al. 2012). Multiple blood feeding by *Ae. albopictus* was reported in every possible combination from the animals and humans present on La Reunion during the chikungunya epidemic (Delatte et al. 2010). Therefore, the frequency of multiple blood-feeding in *Ae. albopictus* should be further studied in the laboratory and field settings.

Hawley (1988) discussed the phenomenon of multiple feeding in *Ae. albopictus* as a result of his field discovery during gut dissections of gravid host seeking females. Since, then several investigators have dissected the ovaries of this species from their human biting collections and found varying proportions of gravid females. Defining the relationship of parity and number of blood meals is confounded by two factors. These factors have been pointed out by Hawley (1988) and are, 1.) some gravid *Ae. albopictus* develop eggs without blood feeding and 2.) some host seeking gravid individuals may have fed more than once before coming to bite the collector. Fu’s result (1982) from laboratory experiments and Li’s observations from field studies (1991) have provided further evidence that gravid *Ae. albopictus* can take a second blood meal. Hawley (1988) noted that while female Asian tiger mosquitoes can take a second blood meal, it was not necessary for successful egg laying. However, Hawley further commented that if the female did not have enough energy reserves in the larval stage then a second blood meal may be essential for egg development.

**IX. BLOOD MEAL SIZE**

Blood meal size is dependent upon mosquito strain, body size, age, host species and defense behavior, and environmental conditions (Klowden 1988, Xue & Edman 1991). The mean blood meal size in a Malaysian strain of *Ae. albopictus* was 2.6 mg (range 1.5-4.2mg) (from Hawley 1988). In a Vietnam strain, blood meal size was 0.2 mg to 2.5 mg (Hien 1976) and in a Chinese strain 1.23-1.83 mg (Fu 1990). Blood meal size from a Japanese strain was 1.2-1.6 ul on unrestrained dogs with 45.2-64.3% of the mosquitoes engorging (Konishi 1989, Konishi and Yamanishi 1984). Blood meal size in *Ae. albopictus* also affects host seeking activity. Barnard and Xue (2009) state that there was a curvilinear relationship between blood meal volume in partially fed *Ae. albopictus* and host avidity with a threshold between 0.8 and 1.0 mg that reduced host seeking.

**X. ARTIFICIAL BLOOD FEEDING**

Laboratory colonization of *Ae. albopictus* is important and necessary for providing research material for a variety of R&D projects. In addition, mass rearing of this species for use in conventional sterile insect release or RIDL (release of insects carrying a dominant lethal) programs for population replacement require a different mass feeding system for adult to egg production. Currently, colonization of any blood feeding mosquito
requires vertebrate blood to optimize egg production. Wind tunnel studies have shown that carbon dioxide, water vapor, warmth, and adenosine triphosphate (ATP) as stimuli that induce host seeking and blood feeding activity (Klun et al. 2013). Generally, *Ae. albopictus* is a difficult species to rear when using artificial techniques (Lyski et al. 2011). However, this has not deterred a variety of workers from trying. Usually sausage casings, or lambskin condoms containing warm bovine, or other animal, blood have been used for artificially feeding several species of mosquitoes, including *Ae. albopictus*. Interestingly, there are instances this mosquito species has preferred sausage casings over lambskin. Ultrastructural analysis revealed that sausage casings have a textured surface while lambskin membranes do not.

Parafilm membrane stretched and pressed into fiberglass window screen to form a packet for holding warmed blood to feed *Ae. albopictus* colonies have also been used successfully by Tseng (2003). Ooi et al (2005) found that cattle skin was the most favorable membrane to use after comparative tests with the skin of chicken, fish, and salt sausage. A 32-46% increase in blood feeding was observed when *Ae. albopictus* were fed using horizontal or vertical blood packets (Lyski et al. 2011). Also, use of bovine collagen sausage membranes in a vertical feeding position will increase the number of engorged females and may be an additional factor influencing feeding success of *Ae. albopictus* (Lyski et al. 2011). A novel system by Deng et al. (2012) consisted of a collagen membrane casing filled with pathogen-free guinea pig blood warmed by a heating device that yielded the same fecundity, survival rate, and egg hatchability when compared with live guinea pigs. Luo (2014) also used this method and found that *Ae. albopictus* fed on whole pig blood with ATP achieved an engorgement rate of 84% compared with 51% from live mice.

Other studies have shown that blood serum and bovine serum albumin, not hemoglobin, may replace vertebrate blood in artificial diets for mass mosquito rearing (Gonzales et al. 2015). However, a recent blood-free protein diet, using a membrane feeding system, was evaluated by Pitts (2014). In that study, *Ae. albopictus* accepted the diet and produced eggs in greater numbers than cohort females fed with whole human blood. This implied that a readily available blood free diet could be utilized in the laboratory for rearing this species. Elimination of handling blood, reduced animal cost, and consistency of diet should potentially be advantageous to mosquito rearing and mass rearing facilities (Pitts 2104).

**XI. OTHER INFLUENCING FACTORS**

Host seeking and blood feeding behavior in *Ae. albopictus* is a complex behavioral process. Friend and Smith (1977) have previously reviewed several factors affecting feeding by bloodsucking insects. Since that time, several additional studies have been conducted on this species and are as follows. Host seeking and blood feeding in *Ae. albopictus* is influenced by human physiological differences, host species, and host defensive behavior. Shirai et al. (2004) reported that human subjects with blood group O attracted more Asian tiger mosquitoes than other blood groups (i.e. B, AB, and A) but was only significantly more attractive than blood group.

Xue (unpubl) found that host defensive behavior significantly affected blood feeding success in *Ae. albopictus* where the percentage of blood-feeding mosquitoes on a restrained host (chicken) was greater than an unrestrained one in laboratory studies (Table 3). Also, Xue and Barnard (1996) found that host attacking behavior of Asian tiger mosquitoes was influenced by age and body size. Large bodied, older females exhibited a higher frequency of biting humans than did younger, small body ones.

Temperature remains a main factor influencing host seeking and blood feeding activity in *Ae. albopictus*. A Chinese strain of this species will feed in a temperature range of 12-35°C in the laboratory. Fu (1990) found that feeding frequency was increased when temperature increased, but a thermal threshold was
met at 35°C that decreased feeding. The USDA, Gainesville, Florida strain of *Ae. albopictus* showed, in laboratory studies, that blood-feeding increased as temperature increased (Table 2). Similar host seeking activity occurred in a Shanghai strain at 17-27°C (Huang and Gao 1991a). Conversely, a seasonal reduction in emergence of host seeking females was observed at a minimum threshold temperature of 13°C in northern Italy (Roiz et al. 2010).

In conclusion, host seeking and blood feeding in *Ae. albopictus* are two of the most important, and exceedingly complex, behaviors that this, or any, mosquito species possess. Both behaviors are influenced by many factors whether they are studied in the field or laboratory. Understanding these processes and identifying the weak links in these relationships, whether it is nuisance and/or disease transmission, will result in more effective management of this species in the future.

### XII. ACKNOWLEDGEMENTS

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### XIII. REFERENCES CITED


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ABSTRACT. Arbovirus surveillance is a core function of the Anastasia Mosquito Control District in St. Johns County, Florida. This agency largely relies on the use of sentinel chicken flocks to monitor annual incidence of arbovirus transmission. Environmental and arbovirus surveillance data for the years 2008-2014 were analyzed. Total monthly rainfall and mean monthly temperature were found to have significant relationships with eastern equine encephalitis seroconversions in sentinel chicken flocks, while only temperature was found to have a significant influence on West Nile virus seroconversion. The identification of environmental and temporal patterns that influence transmission of arboviruses is vital to the formation of effective vector management programs for the District.

Key Words. West Nile virus, eastern equine encephalitis virus, sentinel chicken, vector mosquitoes

I. INTRODUCTION

St. Johns County lies in northeastern Florida with a total area of 1,588 km² (609 sq. miles) and is located between the St. Johns River to the west and Atlantic Ocean to the east. As a result, there is great diversity in salt and fresh water habitats throughout the County that provides developmental habitats for approximately 42 mosquito species. Eleven of these mosquito species are known vectors of mosquito-borne pathogens. Within this jurisdiction, the Anastasia Mosquito Control District (AMCD) oversees the entire county’s mosquito surveillance and control programs with the goals of alleviating the risk of disease transmission and mitigating disease burden. In the last decade, local concerns have increased over mosquito-borne diseases since the first record of West Nile virus (WNV) in 1999 in New York (Hayes et al., 2005) and its eventual spread to St. Augustine in 2001 (Connelly et al. 2012). In addition to public health concerns, the presence of mosquito-borne diseases can have significant economic impact. St. Johns County’s local economy is primarily driven by tourism. However, in spite of the recent national economic downturn, the county is considered one of the fastest growing areas in the United States (SJC TDC, 2015). In light of this fact, AMCD continues to stress the importance of their arbovirus surveillance program as a critical part of control activities that provides economic and public health benefits for the county.

Florida mosquito control arbovirus surveillance, as administered by most local programs, continues to serve as an early warning system for mosquito-borne viral diseases. This program is part of the “Florida Sentinel Chicken Arbovirus Surveillance Network” established in 1978 (O’Bryan and Jefferson, 1991). The Surveillance Network is based on using sentinel chicken flocks to monitor epizootic virus transmission by infected mosquitoes. Anastasia Mosquito Control District participates in this program where data from chicken viral seroconversion rates, together with mosquito surveillance data, provide local public health and mosquito control officials the needed information to assess the frequency and intensity of epizootic virus transmission. Moreover, consistent and appropriate mosquito collection data, coupled with ar-
boviral surveillance, provides the necessary justification for the application of vector control measures for AMCD. Previously, Xue and Qualls (2008) reported arbovirus surveillance results for St. Johns County from 2001 through 2007. In this report, we present the results and analysis of AMCD’s mosquito arbovirus surveillance program for West Nile (WNV), eastern equine encephalitis (EEEV), and Highlands J (HJ) viral diseases in St. Johns County from 2008 through 2014.

II. MATERIALS AND METHODS

Sentinel Chicken Surveillance. Sixty 21-week old chickens were distributed over 10 locations in the county at the beginning of April through December every year to monitor arbovirus activity. Approximately 2.0 ml of blood was taken from each chicken’s wing vein once a week. Blood samples were kept in labeled vacutainers (Fisher Scientific) and transported back to the laboratory at the AMCD Base Station in St. Augustine Beach where they were centrifuged at 4,375 RPM for 15 minutes. Samples were then placed in a labeled and sealed plastic bag, shipped to the Florida State Department of Health (FDOH) Virus Laboratory in Tampa, Florida and tested for WN, EEE, and HJ viruses. Blood samples were sent to the Laboratory on a Monday and results reported to the District by the end of the same week. Once a chicken seroconverted it was removed, destroyed, and replaced with a new bird at its respective location.

Mosquito Surveillance. Population abundance of adult mosquito vectors were monitored by AMCD using CDC light traps (John W. Hock Company, Gainesville, FL) baited with octenol (BioSensory, Inc., Putnam, CT) at 41 permanent locations in the County. Traps were placed outdoors at the beginning of March through the last week of November. Traps were suspended 1 m above ground surface by a shepherd’s hook and operated for 18-20 h using a 12-v battery. Mosquito collections were transported from the field to the AMCD facility and identified to species using the taxonomic keys of Darsie and Ward (2005). Monthly mean rainfall and temperature were obtained for the time period 2008-2014 from NOAA weather station records (http://www.weathersource.com).

Human Surveillance. Human blood samples obtained by local physicians, and other health related professionals, were sent to the FDOH Virus Laboratory in Tampa and tested for WNV, EEE, and Saint Louis encephalitis virus. Positive results were shared by St. Johns County Department of Health with AMCD to determine appropriate local vector control measures.

Other Animal Surveillance. Dead birds reported by local residents to AMCD were referred to the St. Johns County Health Department who sent the remains to the FDOH Tampa Laboratory for detection of WNV. Local veterinary reports and clinical samples from horses and dogs with suspected WNV or EEE infections were tested by the Florida Department of Agricultural and Consumer Services (FDACS) Arbovirus Laboratory in Kissimmee, Florida. This agency shared test results with St. Johns County FDOH and AMCD.

Statistical analysis. Simple logistic regressions were used to determine if significant correlations (\(\alpha=0.05\)) existed between sentinel chicken seroconversion events, mean monthly temperature, and total monthly rainfall using the statistical software program R (ver. 3.13) (R Core Team 2012).

III. RESULTS AND DISCUSSION

A total of 389,899 mosquitoes were collected from CDC light traps from 2008 to 2014 with an average of 5,026 mosquitoes collected per site annually. Forty mosquito species were collected during the study period, including Culiseta melanura Coquillett, Culex nigripalpus Theobald, and Cx. quinquefasciatus Say, the known vectors of EEEV, HJ virus, SLE, or WNV.

The only human case of arbovirus infection in St. Johns County from 2008-2014 was a WNV asymptomatic blood donor reported by FDOH in September 2014. During 2008-2014 there were 184 positive WNV, 113 positive EEE, and 34 positive HJ sentinel chickens reported within the County (Fig. 1). No
arboviral positive dead birds were reported during this time period. There were 5 confirmed EEE horse cases from 2008 to 2014.

West Nile virus seropositive sentinel chickens were reported from May through December with the majority of cases occurring in September (Fig. 2). Eastern equine encephalitis seropositive sentinel chickens were reported from March through November with most seroconversions occurring
in June (Fig. 2). Highlands Jay infection of sentinel chicken flocks was recorded from April through September. Most seroconversions occurred during July. Eastern equine encephalitis horse cases occurred in March through July.

The average rainfall from 2008 to 2014 was 116.9 cm. In 2009, the average rainfall was 155.1 cm, which was the most rain received in a year during this 7 year period. Total monthly rainfall did have a significantly positive relationship with EEE serocon rates in sentinel chicken flocks ($\beta = 0.09$, $SE = 0.04$, $p = 0.028$, $\alpha = 0.05$). It appeared that rainfall may have had a slight negative correlation with WNV ($\beta = -0.07$, $SE = 0.04$, $p = 0.06$, $\alpha = 0.05$) and HJ ($\beta = -0.01$, $SE = 0.06$, $p = 0.82$, $\alpha = 0.05$) seroconversion in sentinel flocks but these relationships were not statistically significant.

The average temperature from 2008 to 2014 was 21.0°C. The highest monthly average temperature was recorded at 21.7°C in 2012. For the 2008-2014 time period, the incidence of WNV in sentinel chickens was negatively correlated with average monthly temperature ($\beta = -0.13$, $SE = 0.02$, $p = 1.78 \cdot 10^{-8}$, $\alpha = 0.05$) while EEE seroconversions were positively correlated with temperature ($\beta = 0.11$, $SE = 0.02$, $p = 4.71 \cdot 10^{-6}$, $\alpha = 0.05$). There was no significant relationship between HJ and temperature ($\beta = 0.07$, $SE = 0.04$, $p = 0.05$, $\alpha = 0.05$).

Arbovirus transmission is influenced by a complex system of environmental, host, and vector interactions (Tabachnick, 2010). Despite the complex nature of this system, the combination of surveillance methods used by AMCD provides the basic framework necessary to protect the health and well being of citizens and tourists alike. However, we suggest that future agency surveillance and geographical sampling resolution can be strengthened through the adoption of novel mosquito arbovirus surveillance technologies incorporating geographic information systems (GIS) and molecular diagnostics.

### IV. ACKNOWLEDGEMENTS

We thank all employees of AMCD and former employees, Whitney Qualls and Ali Fulcher, who partially coordinated and participated in this program. We are thankful for the support from the property owners who allowed us to use their property to place the sentinel chickens.

### VI. REFERENCES CITED


SPATIAL ANALYSIS OF ARBOVIRUS TRANSMISSION IN ST. JOHNS COUNTY, FLORIDA

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ABSTRACT. The arbovirus surveillance program conducted by Anastasia Mosquito Control District is an early warning monitoring system designed to identify the factors affecting the transmission risk of vector borne diseases. The sporadic frequency and intensity of eastern equine encephalitis virus (EEEV) and West Nile virus (WNV) transmission in St. Johns County prompted the use of spatial pattern analysis to understand the geographic distribution of both diseases in the County using Geographic Information System (GIS) technology. The significance of GIS application in surveillance programs stem from its potential to determine the spatial distribution patterns and directional trends of diseases and to produce transmission probability risk maps. Data from 2008-2014 on frequency and intensity of EEEV, WNV sentinel chicken seroconversions and subsequent mosquito vector abundance of Culiseta melanura, Culex nigripalpus, and Cx. quinquefasciatus were used in analyses. Geographically, mosquito vector density and frequency/intensity of disease transmission was randomly distributed. This was also confirmed by logistical analysis that indicated a significantly reduced dependency of EEEV and WNV chicken serconversion records with vector density.

Key Words. eastern equine encephalitis virus, West Nile virus, Culiseta melanura, Culex nigripalpus, Culex quinquefasciatus, Geographic Information System

I. INTRODUCTION

In the past decade, the adoption of Geographic Information System (GIS) technologies by public health agencies has promoted an increased awareness of the spatial and temporal distributions of disease. A powerful tool in the management of community level diseases, geospatial methods have been successfully used to model disease transmission dynamics and produce spatial predictive maps of disease distribution (Dambach et al. 2012). Geographic Information Systems have also proven useful for studying the spatial and temporal distribution patterns of mosquito-borne diseases and their vectors (Kulkarni et al. 2010, Abdel-Dayem et al. 2012, Sallam et al. 2013). The interactions between environmental variables, hosts, and disease vectors are often complex, so the ability to accurately model these parameters at the local level would provide valuable information for public health management decisions, policy formation, surveillance, and disease mitigation.

The Anastasia Mosquito Control District (AMCD) currently provides all mosquito surveillance and control in St. Johns County, Florida, with the goals of mitigating disease burden and minimizing transmission risk. St. Johns County lies in the northeastern part of Florida, USA with a total area of 1,588 km² (609 sq. miles). The County is positioned between the St. Johns River to the west and the Atlantic Ocean to the east (Figure 1). As a result, there is great diversity in saltwater,
freshwater, and vegetative habitat features throughout the County, providing developmental habitats for about 43 mosquito species (Naranjo et al. 2014). Eleven of these species are known vectors of several pathogens, notably arthropod-borne viruses (Lord and Day 2001, Sardelis et al. 2001, Blackmore et al. 2003, Cupp et al. 2003). Public health con-

Figure 1. *Culex quinquefasciatus* collection sites within 5 km buffer zones around sentinel chicken traps.
cern has continued to surface over mosquito-borne diseases since the first domestic case of West Nile virus (WNV) was recorded in New York in 1999 (Hayes et al. 2005). West Nile virus was first reported in St. Johns County in 2001 (Connelly et al. 2012).

Arbovirus surveillance in Florida serves as an early warning system for potential outbreaks of mosquito-borne viral diseases. Established in 1978, the Arbovirus Surveillance Network is the primary system for monitoring mosquito-borne diseases in the state of Florida (O’Bryan and Jefferson 1991). This network is largely reliant on the use of sentinel chicken flocks to monitor endemic virus transmission. In St. Johns County, sentinel chicken arboviral surveillance is a core function of AMCD. Sentinel chicken seroconversion records and mosquito surveillance data are collected by the District that, in turn, aids public health and mosquito control officials in assessing the incidence and transmission risk of endemic virus transmission.

Data driven conclusions drawn from surveillance data provide the necessary justification for the application of larvicides and adulticides. Prior to our study, there has been no attempt to quantify the spatial distribution of WNV and EEEV in St. Johns County. In this report, we present a spatial analysis of the AMCD’s mosquito arbovirus surveillance program for EEEV, WNV, and abundance of vectors in St. Johns County for the years 2008–2014. The delineation of geographic distribution patterns and spatial direction trends of both diseases and their vectors may help to gain insight into their spatial characteristics. Indeed, mosquito abundance has been addressed in previous studies by other authors and have been identified as a major predictor in the likelihood of epizootics (Scott et al. 1983, Anderson et al. 1999, Lord and Day 2001). Also, we plan on comparing EEEV and WNV seropositive sentinel chicken records with that of vector population density in order to test the hypothesis that intensity of EEEV and WNV transmission are correlated with the density of the mosquito vectors in St. Johns County.

II. MATERIALS AND METHODS

Sentinel Chicken Surveillance. Sixty, 21-week old chickens were distributed over 12 locations in the County at the beginning of April through December every year during 2008-2014 to monitor arbovirus activity. Approximately 2.0 ml of blood was taken from each chicken’s wing vein once a week. Blood samples were kept in labeled vacutainers (Fisher Scientific) and transported back to the laboratory at the AMCD base station in St. Augustine Beach where they were centrifuged at 4,375 RPM for 15 minutes. Samples were then placed in a labeled and sealed plastic bag, shipped to the Florida State Department of Health (FDOH) Virus Laboratory in Tampa, Florida where blood samples were tested for WNV, and EEEV. Samples were sent to the Lab on Mondays and results reported to AMCD by the end of the same week. Once a chicken seroconverted it was removed, destroyed, and replaced with a new bird at its respective location.

Mosquito Surveillance. Population abundance of adult host seeking mosquito vectors were monitored by AMCD using CDC light traps (John W. Hock Company, Gainesville, FL) baited with dry ice at 68 permanent locations in the County. Traps were placed outdoors at the beginning of March through the last week of November during 2008-2014. Traps were suspended 1 m above ground surface by a shepherd’s hook and operated for 18-20 h using a 12-v battery. Mosquito collections were transported from the field to the AMCD facility for further identification to species level using the taxonomic keys of (Darsie and Ward 2005).

Abundance data of WNV Culex quinquefasciatus Say and Cx. nigripalpus Theobald, and EEEV vectors, Culiseta melanura (Coquillett), were extracted from AMCD field records within a 5 km buffer zone around each sentinel chicken trap using the geoprocessing package in ArcGIS (ver. 10.0). Buffer zones were used as an indication of probable mosquito transmission within their flight range around sentinel chicken traps (DeMeillon 1934, Nayar and Sauerman Jr 1973) (Figures 1-2). In addition, the study area was
categorized into AMCD operational route zones (Figure 3) previously extracted from St. Johns County GIS division. This was done in order to provide data driven guidance risk maps for the operational surveillance and control activities conducted by AMCD field personnel. In addition, these operational route zones were coupled with the risk maps produced in order to accurately identify the focal risk areas.

Figure 2. *Culiseta melanura* collection sites within 5 km buffer zones around sentinel chicken traps.
Spatio-Statistical Analysis. Parameters of EEEV and WNV seroconversion data (frequency and intensity), and mosquito vector density were analyzed using spatial statistics tools in ArcGIS (ver. 10.0). The standard deviational ellipse (SDE) analyses in this package were used to summarize the spatial patterns of the above parameter values around their mean. The SDE creates a polygon to summarize the spatial directional distribu-
tion which may show dispersion, central tendency, and directional trends. Therefore, SDE was used to map the directional distribution trend of mosquito vectors and spread of EEEV and WNV transmission.

Spatial autocorrelation analysis, using Global Moran’s I Index, was used to identify the distribution pattern of mosquito vectors, EEEV, and WNV sentinel chicken seroconversion. The spatial autocorrelation pattern may be random, clustered, or dispersed (Lee and Wong 2001). The distribution pattern is predicted by comparing values of neighboring sampling sites for: 1.) mosquito vector density and 2.) frequency and intensity of EEEV and WNV seroconversion records. A strong positive spatial autocorrelation (clustered) is indicated when the neighboring sampling points have similar values. Whereas, if the neighboring sampling points have dissimilar values (dispersed), then it indicates strong negative spatial autocorrelation. The value of Moran’s I Index is between -1 and 1. The ‘Z’ score value is calculated to test whether the observed clustering or dispersing is significant. When the Z score indicates statistical significance, a positive Moran’s I index indicates tendency toward clustering while a negative value indicates tendency toward dispersion. When the Z score value is not significantly different from 0, there is no spatial autocorrelation and the pattern does not appear to be significantly different from a random distribution. The null hypothesis (Ho) for the current analysis was that there is no spatial clustering of EEEV, WNV with abundance of the mosquito vectors in St. Johns County.

Logistic regression R statistical software (ver. 3.13) was used to assess the relationship between frequency of sentinel chicken seroconversion records and the abundance of their corresponding mosquito vectors (i.e., *Culiseta melanura*, *Cx. nigripalpus*, and *Cx. quinquefasciatus*) sampled within a 5 km buffer zone around the chicken flocks.

### III. RESULTS AND DISCUSSION

From 2008 through 2014 there were 113 and 184 sentinel chicken seroconversions reported from the County for EEEV and WNV antibodies, respectively. A total of 389,899 mosquitoes were collected from CDC light traps with an annual average of 5,026 per site. Forty mosquito species were collected during the study period including EEEV and WNV bridge vectors and other non-anopheline mosquitoes that represented 68.43% of the total mosquitoes collected. *Culiseta melanura*, *Cx. nigripalpus*, and *Cx. quinquefasciatus* represented 1.01%, 5.91%, and 2.72%, respectively of the total non-anopheline mosquitoes collected during the study.

The SDE analysis indicated that density of *Cs. melanura*, *Cx. nigripalpus*, and *Cx. quinquefasciatus* and intensity records of EEEV, WNV were randomly distributed and showed a directional trend about the mean; this is verified by the ellipsoid polygons in Figures 4-6. SDE analysis revealed that a random distribution pattern highlighted the habitat suitability for these mosquito vectors within the ellipsoid polygons. These results were further confirmed by Moran’s I index values where these same parameters were found to be insignificant (*P*>0.05) confirming the findings generating by the SDE polygons. Because we sampled adult host seeking mosquitoes, these individuals were most likely newly emerged from their developmental habitats within the risk polygons. Accordingly, this reflects the probable abundance of larval habitats for these vectors within the polygons. Similarly, extracted data on mosquito vectors around sentinel chicken traps reflected the flight range of these individuals and the availability of their host(s) as a source for blood meals. Although adult host seeking mosquito vectors were collected outside the risk polygons (which may have been infective with either EEEV or WNV) larval habitats within the ellipsoid polygons are worthy of surveillance and should be evaluated for control compared with other areas.

The random distribution of seropositive records of EEEV and WNV indicated virus circulation inside the mosquito vectors rather than virus amplification inside bird host(s). Although the spatial analysis potentially predicted the directional distribution pattern of infective mosquito vectors, and their flight
range, during host seeking, the number of parameters used in this analysis prevented us from highlighting virus amplification inside their reservoir host(s). This limitation is attributed to lack of consistent distribution data on reservoir host(s), which represents the source of infection for uninfected mosquito vectors within their flight range.

Figure 4. Statistical distribution pattern of WNV and *Culex quinquefasciatus* in St. Johns County showing standard deviational ellipsoids.
The GIS spatial analysis predicted high risk areas for mosquito bites for *Cs. melanura*, *Cx. nigripalpus*, and *Cx. quinquefasciatus* that covered 806.67 km² (50.80%), 825.12 km² (51.96%), and 961.16 km² (60.53%) of the total land area of the County, respectively. These areas were found to represent AMCD operational route zones N01-N03, C04-C08, S01-S03 for the three mosquito vectors, respectively. Furthermore, the amount of land under EEEV
and WNV transmission risk from probable infective mosquito vectors represented 45% and 42%, respectively, of the total area of St. Johns County. These risk areas were found to represent operational route zones N02, N03, C04-C08, S01 for EEEV and N02, N03, C04-C09, S01, S02 for WNV. Overlapping between polygons produced for high transmission risk areas shown in Figs. 4-6 show the likelihood of probable association between pathogen,
vector, and reservoir host. In this context, the overlapping areas under risk of probable infective mosquito bites should be targeted for EEEV and WNV surveillance and control activities rather than the entire county.

The predicted distribution developed by SDE polygons demonstrated the relative expanded distribution pattern of *Cx. quinquefasciatus* compared with the other two vectors. This reflected either a wide flight range of this mosquito or the availability of suitable larval habitats and/or availability of hosts as a blood meal source. However, the parameters used in our analysis prevented us from addressing actual flight range away from their larval habitats. Further investigations are needed to include more parameters on larval developmental sites in proximity to wild reservoir host populations.

In previous studies, the density of mosquito vectors played a major role in predicting the transmission of arboviral diseases (Scott et al. 1983, Anderson et al. 1999, Lord and Day 2001). We found that eastern equine encephalitis virus seroconversion rates were significantly correlated with the density of *Cs. melanura* ($\beta = 0.001$, $SE = 0.000$, $p = 1.10^{-7}$, $\alpha = 0.05$) (Figure 7). Also, the WNV serocons were positively correlated with the abundance of *Cx. quinquefasciatus* ($\beta = -0.002$, $SE = 0.000$, $p = 5.26 \times 10^{-10}$, $\alpha = 0.05$) and *Cx. nigripalpus* ($\beta = 0.003$, $SE = 0.000$, $p = 1.10^{-14}$, $\alpha = 0.05$) (Figure 8). Surprisingly, even though correlations were significant (i.e. $P<0.05$) between seroconversion rates and vector abundance, in our study, the dependency of seroconversion on mosquito vector density was considerably reduced because both values of ($\beta$) and ($p$) were close to zero which indicated that those relationships were random. These results highlight the important contribution that other culicine bridge vector(s) may have in the disease transmission cycle. Moreover, our findings

![Figure 7](image_url). Annual abundance of eastern equine encephalitis virus seropositive sentinel chicken records, *Culiseta melanura* density during 2008-2014.
were confirmed by the evident slight shifting in SDE polygons between mosquito vectors and their diseases (Figures 4-6). Previous studies confirmed that Cx. melanura, Cx. nigripalpus, and Cx. quinquefasciatus are competent vectors in transmitting EEEV and WNV (Anderson et al. 1999, Blackmore et al. 2003, Cupp et al. 2003) in comparison with other mosquito vectors. However, the contribution of other culicine bridge vectors such as Cx. restuans Theobald, Cx. erraticus (Dyar and Knab), Aedes vexans (Meigen), and Uranotaenia lowi Theobald, for EEEV transmission (Chamberlain et al. 1954, Cupp et al. 2003, Cohen et al. 2009, Estep et al. 2013) and Cx. salinarius Coquillett for WNV (Sardelis et al. 2001, Blackmore et al. 2003) should be considered. The contribution of other bridging vectors exacerbates the complexity of virus circulation in nature. The variation of feeding preference by bridge vectors may sustain virus circulation in other reservoir host(s) such as amphibians and possibly reptiles (Cupp et al. 2003, Cupp et al. 2004).

In summary, AMCD arbovirus surveillance showed that the overall distribution of EEEV, WNV and their mosquito vectors were confined to certain areas of St. Johns County. This may be attributed to possible ecological niches for mosquito vectors, seasonal abundance of animal reservoirs such as birds, amphibians, reptiles, and possible bridge vector(s). Additional information is needed from these parameters in order to shed light on virus amplification and characterize various habitats suitable for mosquito vectors and reservoir host(s). Although some disease surveillance methods provide more information than others, combining traditional operational surveillance programs with GIS technologies and modeling packages will enhance the ability of public health agencies to maximize the benefits of practical application of disease surveillance data. Developing real time data driven risk maps will help in the decision making process by prioritizing control activities, at the District level, while increasing control effectiveness, plus reducing labor and associated cost especially during periods of arboviral outbreaks.

IV. ACKNOWLEDGEMENTS

We would like to express our deep gratitude to Mr. Richard Weaver for his consistent help in supporting us with the necessary data for the current work. Also, we extend
our thanks to all employees in AMCD and former employees especially Dr. Whitney Qualls and Ali Fulcher. Moreover, authors extend their gratitude to the two anonymous reviewers for their valuable comments that potentially helped in improving the readability and the significance of the current investigation.

VI. REFERENCES CITED


RELATIONSHIP BETWEEN CITIZEN KNOWLEDGE, VEGETATION COVERAGE, AND FREQUENCY OF REQUESTS FOR MOSQUITO CONTROL SERVICE IN ST. JOHNS COUNTY, FLORIDA

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2Anastasia Mosquito Control District 500 Old Beach road, St. Augustine, FL 32080
3Leonard and Jayne Abess Center for Ecosystem Science and Policy University of Miami, Coral Gables, FL 33146

ABSTRACT. Customer service requests play an important role in mosquito control programs by providing mosquito surveillance information and by allowing the opportunity for inspectors to educate the public on mosquitoes and mosquito borne diseases. In this study, we assessed differences in knowledge, attitude, mosquito control practices (KAP), and vegetation characteristics between frequent service requesters and non-frequent service requesters by conducting paper, vegetation, and entomological surveys. There were no significant differences in customer knowledge about mosquitoes between the two groups. However, there was a general lack of knowledge about mosquito resting and sugar feeding behavior in >90% of participants. Perceptions of mosquito infestation and vegetative coverage were determined to be driving factors for frequent service requests. Frequent service requesters reported being bothered or bitten by mosquitoes a few days a week or daily more often than non-frequent; demonstrating that perceptions of the severity of mosquito infestation are associated with service request volume. Vegetation coverage on residential properties of frequent service requesters was 13% higher than non-frequent service requesters and identified vegetation coverage as another potential factor driving service request volume. We conclude that future education programs should include information on the associations of plants with mosquito behavior, such as resting and sugar feeding, to better inform the public and aid in increasing their knowledge base as they relate to homeowner control practices.

Key Words. Service request; Vector Control Management System; KAP survey

I. INTRODUCTION

Although the majority of mosquito-related issues in the United States arise from them being a nuisance, there remains an imminent threat of the emergence and re-emergence of mosquito-borne diseases. In 2002, 4,156 cases of the West Nile Virus were reported in the United States; in 2005, 25 cases of dengue fever were reported in Texas; and between 2009 and 2010, 90 cases of dengue fever cases in Key West, Florida were reported (O’Leary et al. 2004; CDC 2007, CDC 2013). By the end of 2014, there have been 11 locally acquired chikungunya cases in Florida, and though it rarely results in death, the disease can be debilitating to those infected (CDC 2014).

Mosquito control programs, like Anastasia Mosquito Control District (AMCD) of St. Johns County, Florida, use surveillance methods to monitor nuisance and vector
capable mosquitoes to reduce and prevent the spread of mosquito-borne diseases (Day and Shaman 2011). Despite the efforts of these programs, an increase in the incidence of mosquito-borne diseases is still of major public health concern. It is imperative that these programs continue to conduct applied research, as well as continued surveillance to produce new or improved approaches for mosquito control.

The District uses the Vector Control Management System (VCMS) to manage service requests made by residents of St. Johns County. Service requests entered into VCMS play a major role in supporting surveillance efforts (AMCD 2013, Weaver et al. 2013). This method of surveillance has proven to be successful as there is a correlation between the volume of service requests received and the density of mosquitoes (Morris and Clanton 1981, 1992). The software allows for service requests to be linked with mosquito abundance, along with information of their location in the county. The VCMS system relies on people to make service requests; however, some residents make more frequent requests than others. Moreover, there seems to be a disconnect in the knowledge, attitude, and control practices between residents that call in service requests frequently and residents who do not (AMCD staff pers. comm.).

In addition, over the last few years, AMCD staff has observed that residents who frequently put in service requests seem to have more vegetation on their property than residents without service requests (AMCD staff pers. comm.). The density of vegetation on residential properties is important because mosquitoes rely on sugar from plants for energy (Hocking 1953, Nayar and Van Handel 1971, Van Handel 1965) and vegetation for resting sites (Allan et al 2009). Energy gained from plants is used to support their host seeking behavior in flight and fecundity, increasing the probability of disease transmission (Muller et al. 2011, Yuval 1992). Therefore, the method in which a residential property owner manages his/her agricultural landscape is correlated with mosquito population abundance (Day and Shaman 2011).

Understanding residents’ knowledge and attitude, toward mosquito behavior and ecology on residential properties is essential for developing and/or improving mosquito control approaches. Therefore, the purpose of this study is to: 1) determine if there are differences in knowledge, attitude, and control practice of residents in regards to mosquitoes between residents; 2) determine if service requests are related to vegetation on properties; 3) identify major vegetation density on residential properties of service requesters; and 4) determine the major species of mosquitoes and abundance in residential properties. This study will provide feedback to AMCD and lessons learned can be used as a tool by the District to continue to protect public health by revising educational materials, and serving as a catalyst for discussion on future studies involving vegetation, mosquito behavior, and control.

II. MATERIALS AND METHODS

Study site selection and sampling: Data collection was conducted in St. Johns County, Florida between June 3 and July 31, 2013. St. Johns County, with a population of approximately 202,000, covers 609 square miles of northeastern Florida (US Census 2010, Weaver et al. 2013). The VCMS is an integrated database and mobile field data collection system used by AMCD for mosquito control (Weaver et al. 2013). The software assists with management of the District’s mosquito borne disease and surveillance data; integrating mosquito management activities (service requests, inspection, and spraying); and monitoring employee activities. Using the VCMS program, four years’ worth of service requests was assessed to categorize and identify residential properties. Service requests were grouped as 1, 2, 3, 4, and >4 requests by household within each year. The data was filtered by deleting multiple repeat entries that were not different requests, this allowed for a more accurate description of the frequency of calls over the past four years.
Residential properties that had frequent service requests were selectively sampled from the VCMS database. Requests had to meet three criteria: 1) be a residential property in which an occupant has made >3 requests in 2012; 2) multiple requests had to have the same address but different dates of entry; and 3) properties could not be farm lands, apartment complexes, or greater than 43,560 sq ft. If occupants were not available at the pre-selected household, then the next pre-selected household was visited until consent was granted.

Residential properties that were not frequent service requesters or non-service requesters, were selected at random as control sites. Control sites also had to meet three criteria: 1) residential properties had to be within the same neighborhood as a study site, either on the same street or the next street over; 2) they were not identified as having >3 service requests a year between 2009-2012; and 3) properties could not be farm lands, apartment complexes, or a larger than ≥43,560 sq ft. If occupants of a household were not available or refused to participate, neighboring houses were visited until consent was granted.

Study sites identified with these criteria and sampling techniques resulted in 24 residential homes within 4 zip codes: 32080, 32082, 32084, and 32086. Of these study sites, six houses were randomly selected from each zip code, three of which were identified as frequent service requests from the VCMS database and three randomly selected control sites. All houses were visited during the evening hours (4pm-6pm) for 1.5 weeks: July 8-16, 2013.

Paper-based KAP survey. A member of the research team administered a knowledge, attitude, and practice (KAP) questionnaire to one consenting adult (≥18 years old) from each household. The questionnaire was designed to elicit resident's knowledge on mosquito behavior, mosquito-borne disease, attitudes towards and practices used in mosquito control. Their responses were assumed representative of the household. All questionnaires were asked face-to-face and most questions were asked as open-ended questions. No demographic information was collected, as it was not necessary for the completion of this study.

Vegetation survey. After each questionnaire was administered, consent was requested to survey their property and measure vegetation growth. This measurement was taken with the use of a 1,000 ft tape-measuring wheel (#PSMW48, Lufkin, Sparks, MD). All vegetation above ground level was measured by taking the circumference or perimeter and calculating its area in square feet. Tree canopies and grass coverage were not measured as investigators were only interested in non-grass vegetation that covered the soil surface. Circumference of trees was measured at their base and included in the survey. To calculate vegetation coverage of the property, measurements were summed and divided by the total square footage of the property. Square footage of each property was obtained from the St. Johns County Property Appraiser’s Office website. Photos of vegetation were taken on each property. Frequently occurring plants on each property were identified by Ali Fulcher, at AMCD, and Keith Fuller, at the St. Johns County Agriculture Center.

Entomological survey: In addition to the vegetation survey, consent to check participants’ yards for adult mosquitoes and mosquito larval habitats were also obtained upon completion of the questionnaire. To determine the presence of adult mosquitoes, landing rate counts (LRC) were conducted by volunteers. Landing rate counts consisted of volunteers exposing the surface from their elbow to their hand, and counting how many mosquitoes landed, within three minutes at each property. Mosquitoes were allowed to land, but were not allowed to take a blood meal. Landing rate counts were conducted in areas identified as possible resting areas (shaded vegetated areas).

Each property was also checked for containers or plants with standing water for the presence or absence of larvae or pupae. Larvae and pupae were not collected. Containers found with standing water were emptied by surveyors. Adult mosquitoes were collected with the use of a BioGents sentinel (BGS) trap with BGS lure and were set up for 24 hrs
on each property. The BGS trap was chosen due to its ability to attract various container ovipositing mosquitoes such as *Aedes* spp. and *Culex* spp. Mosquito collections were taken within a day or two of administering the questionnaire. Collected mosquitoes were brought back to the lab and kept frozen in a standard freezer until they could be sexed, identified to species, and counted.

**Data analysis.** SPSS (ver. 21) was used to assess differences in vegetation coverage, knowledge, attitude, and practice between frequent and non-frequent service requesters. Chi-square tests were used to test for statistical significance. Mean differences of vegetation coverage, mosquito collection, knowledge, and practice scores were calculated using an independent t-test.

### III. RESULTS

#### Paper-based survey.
Twenty-four participants completed the survey, 12 of which were from households that had requested services frequently and 12 from households that had not requested services frequently. Each participant answered every question on the survey.

**Knowledge.** An equal number of frequent service requesters and non-frequent service requesters (4 each) scored as being very knowledgeable of mosquitoes. The remaining frequent and non-frequent service requesters (8 each) scored as not being very knowledgeable about mosquitoes (Table 1). Participants who rated their propensity of knowledge as 4-5, on average, had a higher knowledge score than those who self-rated as being not very knowledgeable.

Overall, 92% of the participants were able to identify at least one source of mosquito larval habitat such as standing water, marshes, swamps, or damp/moist areas. More than 75% of the participants were able to list at least one mosquito-borne disease, such as malaria, dengue, West Nile virus, eastern equine encephalitis. When asked where mosquitoes generally rested as adults, only 25% of participants were able to identify vegetation as a resting area. Specific knowledge of non-blood meal sources for mosquitoes was low, with only one responder reporting knowledge that plants are a meal source for mosquitoes. There were no significant differences between non-frequent service requesters and frequent service requesters in overall knowledge scores or specific knowledge about mosquito behavior and disease (Table 1).

**Attitude.** A majority of the participants reported being bothered or bitten by mosquitoes daily or a few days a week with 71% reporting being bothered or bitten daily during the summer months (Table 2). An additional 8% reported being bothered or bitten a few days a week. There was a significant association ($\chi^2, p = 0.019$) between service request status and reporting how often they are bothered or bitten by mosquitoes. On average, frequent service requesters reported being bothered or bitten by mosquitoes more often than non-frequent service requesters. When asked where they were more likely to be bothered by mosquitoes, over 80% of the

<table>
<thead>
<tr>
<th>Question</th>
<th>Non-frequent service requesters</th>
<th>Frequent service requesters</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Mosquito breeding sources</td>
<td>10</td>
<td>83.3</td>
<td>12</td>
</tr>
<tr>
<td>Mosquito resting area</td>
<td>2</td>
<td>16.7</td>
<td>4</td>
</tr>
<tr>
<td>Mosquito meal sources</td>
<td>1</td>
<td>8.3</td>
<td>0</td>
</tr>
<tr>
<td>Mosquito sugar feed on plants</td>
<td>1</td>
<td>8.3</td>
<td>0</td>
</tr>
<tr>
<td>Mosquito borne diseases</td>
<td>10</td>
<td>83.3</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average Knowledge Score</th>
<th>Non-frequent service requesters</th>
<th>Frequent service requesters</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>2.00 (1.04)</td>
<td>2.08 (0.67)</td>
<td>2.04 (0.85)</td>
</tr>
</tbody>
</table>
participants reported being bothered outdoors on their property. When asked what time of the day they were outdoors on their property, most participants reported being outdoors mostly in the evening hours and multiple times to most of the day (80%). More than 80% of participants reported great concern towards reducing mosquito larval habitats, scoring 4 or 5 on a five point scale (Table 2). On average, frequent service requesters reported spending more time outdoors (22.75 hours) than non-frequent service requesters (18.67 hours).

**Practice.** Most participants reported that they did something to reduce larval habitats, including all of the frequent service requesters (Table 3). Participants varied in their responses on the measures they take to reduce larval habitats, with emptying standing water as one of the most frequent methods used. Participants also varied in their responses on the personal protection measures used to protect themselves and their family, with the use of mosquito repellent as one of the most frequently used methods. On average, frequent service requesters had a significantly higher practice score \((p = 0.023)\) than non-frequent service requesters.

**Vegetation survey.** Vegetation type and coverage varied between residential properties (Table 4). While not significant, frequent service requester properties had 13% greater vegetation coverage than non-frequent services requesters (Figure 1). When comparing mean differences between these two groups within each zip code, no significant differences were found in 3 out of the 4 zip codes with. In zip code 32080 there was a significant \((p < 0.05)\) difference in mean vegetation coverage between the two groups (Figure 2). The more vegetation coverage the more service requests.

**Entomological survey.** There were no significant differences between frequent and non-frequent service requesters in the average number of mosquitoes trapped (Figure 4). The majority of the mosquitoes caught in the BGS traps were container ovipositing mosquitoes, of which 70% were *Aedes albopictus* (Skuse). Average landing rate counts, conducted 3 months post-initial survey, were less than or equal to one, with the exception of one home, which had an average count of 5.7 mosquitoes. Approximately half of the houses surveyed were positive for containers with standing water. However, there were no

### Table 2. Survey mean responses (SD) to open-ended questions about attitude towards mosquitoes.

<table>
<thead>
<tr>
<th>Question</th>
<th>Non-frequent service requesters' n (%)</th>
<th>Frequent service requesters n (%)</th>
<th>Overall n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>How often were you bothered or bitten by mosquitoes?*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily or few days a week</td>
<td>7 (58.3)</td>
<td>12 (100.0)</td>
<td>19 (79.2)</td>
</tr>
<tr>
<td>Few days a month or fewer</td>
<td>5 (41.7)</td>
<td>—</td>
<td>5 (21.8)</td>
</tr>
<tr>
<td>Where on your property you were most likely to be bothered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outside around house</td>
<td>8 (66.7)</td>
<td>12 (100.0)</td>
<td>20 (83.3)</td>
</tr>
<tr>
<td>Inside Home</td>
<td>1 (8.3)</td>
<td>—</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Everywhere</td>
<td>2 (16.7)</td>
<td>—</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Not sure</td>
<td>1 (8.3)</td>
<td>—</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>What time of the day outdoors were you bothered?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>2 (16.7)</td>
<td>1 (8.3)</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Afternoon</td>
<td>1 (8.3)</td>
<td>1 (8.3)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Evening</td>
<td>3 (25.0)</td>
<td>2 (16.7)</td>
<td>5 (21.8)</td>
</tr>
<tr>
<td>Multiple times/All day</td>
<td>6 (50.0)</td>
<td>8 (66.7)</td>
<td>14 (58.3)</td>
</tr>
<tr>
<td>How concerned are you about reducing mosquitoes?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-3 score, not very concerned</td>
<td>2 (16.7)</td>
<td>1 (8.3)</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>4-5 score, very concerned</td>
<td>10 (83.3)</td>
<td>11 (91.7)</td>
<td>21 (87.5)</td>
</tr>
<tr>
<td>Time spent outdoor on property per week</td>
<td>18.67 (13.4)</td>
<td>22.75 (14.6)</td>
<td>20.7 (13.9)</td>
</tr>
</tbody>
</table>

*Percentages are column % out of 12 within each group and out of 24 for overall

*p* significant difference in response between groups \((p < 0.05)\).
significant differences in the presence or absence of containers with standing water between the two groups.

**IV. DISCUSSION**

There were no significant differences in overall or specific knowledge about mosquitoes between the two groups, where most participants reported not being very knowledgeable about mosquitoes. Most residents were able to identify mosquito larval habitats and at least one mosquito-borne disease. However, collectively, there was a lack of knowledge about mosquito resting and feeding behavior with only six individuals correctly identifying some kind of vegetation as a mosquito resting area. Only one individual correctly identified plants as a meal source. The other residents reported that mosquitoes generally rested on humans and animals and that they feed on human and/
or animal blood for their main meal source. The beliefs of the majority of the interviewees, could be due to lack of education and dissemination about mosquito behavior. By reviewing educational materials provided by national and local institutions, it was observed that there was a constant emphasis on disease information and control/prevention practices for the layman that included reducing standing water, wearing protective clothing, and altering daily activities around dawn and dusk. Although these methods have been repeatedly proven to be effective in protecting the public health and the environment (WHO 2014), it is not sufficient in educating the public on mosquito behavior.

As expected, frequent service requesters reported being bothered or bitten by mosquitoes more often than non-frequent service requesters. In addition, frequent service requesters tended to spend more time outdoors and to take a number of measures to protect themselves and their families from nuisance and disease carrying mosquitoes; more so than non-frequent service requesters. This supports their belief that they are bothered or bitten by mosquitoes more often than the non-frequent requesters are bitten.

We found that there was a mean difference of 13% higher vegetation seen in the frequent service request residence, the lack of significance was probably due to the extremely small sample size and/or the method used to measure vegetation coverage on residential properties. Previous work with vegetation coverage utilized a grid method in which each grid size is dependent on the plant shrubs, trees, or flowering. Environ-
mental factors such as seasonality of plants and the density of tree canopies, which may affect the behaviors of mosquitoes, were not taken into consideration (Daubenmire 1959).

Although vegetation types varied across all 24 houses, similarities were seen in the occurrence of a number of plants that can support mosquito development, feeding and resting, such as the *Bromelia* spp., *Polystichum munitum*, *Ruellia* spp., and *Callicarpa* spp. Further studies should investigate mosquito preferences for types of vegetation, as well as their uses for them for feeding or resting. The major species of mosquito caught in BGS traps was *Ae. albopictus*. This maybe due to the anthropogenic changes to landscape vegetation and artificial uncovered containers, in which this species has been proven to thrive in. In addition, the time of year that trapping occurred (i.e. a summer month with high temperature and precipitation) provided ideal abiotic factors for development of *Ae. albopictus* (Paupy et al. 2009).

To our best of knowledge, our study is unique in its assessment of key factors (e.g., KAP and vegetation) driving the volume of mosquito control service requests. We conclude that knowledge and practice did not vary greatly by service request volume, but residents perceptions of how often they are bothered/bitten was a driving factor for service request volume. Overall, residents were proficient in their general knowledge about mosquito larval habitats and disease; however, they lacked knowledge about resting and feeding behavior of adult mosquitoes. To be comprehensive, future educational materials for mosquito control and disease prevention should include this information. Vegetation was not found to be a driving factor for service request calls. Vegetation cannot be ruled out. Previous studies have shown that mosquitoes do rely on vegetation for resting sites and plant sugars for fecundity and flight (Muller et al. 2001, Yuval 1992). Future research should expand the experimental sample size of survey participants for a more accurate analysis of the correlation of vegetation and service requests.

V. ACKNOWLEDGMENTS

Thanks to A. Fulcher, M. Smith, and K. Fuller for their assistance during this study. All human subjects were informed before administering surveys based on the District-approved protocol for using human subjects.

VI. REFERENCES CITED


MOSQUITO SPECIES COMPOSITION AND IMPACT OF TRAPPING SITES ON FLOODWATER MOSQUITOES, Aedes vexans IN XINJIANG, CHINA

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ABSTRACT. Mosquito species composition and impact of mosquito trapping sites on a major species of floodwater mosquito Aedes vexans, was investigated by using Mosquito Magnet Liberty Plus (MMLP) traps baited with octenol in Beiwan, Xinjiang. A total of six trapping sites inside (three) and outside (three) human occupied areas were selected for the study. Six species of mosquitoes, Aedes vexans, Anopheles messeae, Ae. cyprius, Coquillettidia richiardii, Culex modestus, and Cx. pipiens pipiens were collected during the study. The results indicated that Ae. vexans was the major species and represented 97.14% of the total collection. Mosquito Magnet Liberty Plus traps also significantly reduced the number of mosquitoes in the residential areas. We found that MMLP traps were suitable tools for the collection of floodwater mosquitoes in Xinjiang where extremely high floodwater mosquito production occurred as well as provide protection of humans and livestock from host seeking mosquitoes.

Key Words: Mosquito Magnet® Liberty Plus, surveillance, Aedes vexans

I. INTRODUCTION

Beiwan is located near the Chinese border with Kazakhstan and is situated along the flood lands of the Irtysh River, Xinjiang in western China. River water levels rise annually inundating the residential area and adjoining semi-desert land. After the flood water recedes to its normal level, many shallow ponds, lowland puddles, water holes, and swamps become ideal production sites for floodwater mosquitoes. Previous studies, have shown that extremely high floodwater mosquito production occurs creating a serious health threat for humans and livestock (Lu and Liu, 1990; Ma and An, 1994; Liu and Zhang et al., 2005) including potential alphavirus transmission in Xinjiang (Li and Liang et al., 1992; Li and Karabatsos et al., 1995). Therefore, it is of critical importance to determine the species composition and seasonal abundance of floodwater mosquitoes in this region. Furthermore, we wanted to determine if commercial mosquito traps could be used in residential areas to reduce local floodwater mosquito populations in Beiwan, Xinjiang.
located in the transition belt between these two different landscapes.

**Sampling locations.** A total of six trapping sites were selected for the study. Three trapping sites were located along the outside of the dike in the residential area and were: riverside (A site), semi-desert (B site), and a middle zone (C site) between sites A and B. Three trapping sites inside the residential area were located along the dike, riverside (D site), semi-desert (E site), and a middle zone (F site) between sites D and E.

Mosquito Magnet® Liberty Plus traps (MMLP) baited with octenol was provided by the former American Biophysics Corporation, USA. In each trapping site, the same MMLP was operated in the field for two days. Mosquitoes in each trap were collected every eight hours (a total of 6 collections) and brought back to the laboratory for species identification and total counts. Collections were made during the peak flood season (July and August).

**Statistical analysis.** The proportion of each species (Pi) in MMLP collections was calculated as Pi (%) = (Ni*100)/N, where N is total number of mosquitoes and Ni the number of species “i”. Chi square test was performed to compare the Pi of different mosquito species between sites and species (P = 0.05)

### III. RESULTS

A total 251,475 mosquitoes representing six species was collected from MMLPs and included *Aedes vexans* (Meigen), *Ae. cyprius* Ludl., *Anopheles messeae* Falleroni, *Coquillettida richiardii* (Ficalbi), *Culex modestus* Ficalbi, and *Cx. pipiens pipiens* L. (Table 1). Chi square test results revealed that a significant

<table>
<thead>
<tr>
<th>Species</th>
<th>Pi(%)</th>
<th>QTY</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aedes vexans</em></td>
<td>97.14</td>
<td>244,279</td>
</tr>
<tr>
<td><em>Ae. cyprius</em></td>
<td>1.30</td>
<td>3,280</td>
</tr>
<tr>
<td><em>Anopheles messeae</em></td>
<td>0.53</td>
<td>1,342</td>
</tr>
<tr>
<td><em>Coquillettida richiardii</em></td>
<td>0.80</td>
<td>2,007</td>
</tr>
<tr>
<td><em>Culex modestus</em></td>
<td>0.09</td>
<td>229</td>
</tr>
<tr>
<td><em>Cx. pipiens pipiens</em></td>
<td>0.13</td>
<td>338</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100.00</td>
<td>251,475</td>
</tr>
</tbody>
</table>

Pi = proportion of each species; QTY = number of mosquitoes

\[ \chi^2 = 254902, \ p<0.001 \]
statistical difference existed between the proportion of each species ($\chi^2 = 254902$, $p < 0.001$) with *Ae. vexans* as the dominant species (97.14% of total catch). The other 5 species made up about 2.86% of the total collection.

The proportion of *Ae. vexans*, *An. messeae*, *Cx. modestus*, and *Cx. pipiens pipiens* caught from the inside human residential area were higher than those caught outside (Table 2). The proportion of *Ae. cyprius* and *Cq. richiardii* collected from inside human the residential area were lower than those caught from outside the area. Also there was a significant difference between the proportion of mosquito species outside and inside the residential area ($\chi^2 = 16.486$, $p < 0.01$).

There was a significant difference in the proportion of each species between trapping sites inside the residential area ($\chi^2 = 392.86$, $p < 0.001$) (Table 3). *Culex pipiens pipiens* from the D site inside the residential area were higher than the other areas. However, the proportion of *Ae. vexans* from the D site area was lower than those collected from the F site and E site areas. Also, similar proportions of *Ae. vexans*, *Ae. cyprius*, and *An. messeae* were collected from the F site and E site areas. No *Cx. pipiens pipiens* was collected from all three sites.

There was a significant difference in the proportion of each species between the three trapping sites in the outside residential area ($\chi^2 = 6201.936$, $p < 0.001$) (Table 4). *Aedes vexans* and *An. messeae* collected from the C site were lower than those collected from sites A and B; however, the proportion of *Ae. vexans* and *An. messeae* were similar between those 2 latter collection sites. The proportions of *Cx. modestus* and *Cx. pipiens pipiens* collected from the C site area were higher than those from the A site and B site.

### IV. DISCUSSION

*Aedes vexans* is a major nuisance species in northern China (Liang, 1997). Moreover, Japanese B encephalomyelitis virus has been isolated from this species in China (Li and Liang et al., 1992; Zhang and Shi et al., 1999). Our study showed that *Ae. vexans* still was the major species and represented 97% of the total MMLP collections. Because this species is one of the more proliferate floodwater mosquitoes, it poses a great health threat to residents and livestock in this area. Although few numbers of *An. messeae* were collected in MMLPs, this species is a suspected vector of malaria (Tong and Li et al., 1996; Lu, 1999). Additionally, *Cx. modestus* and *Cx. pipiens pipiens* were collected from traps in low abundance and are suspected vectors of West Nile virus (Hayes, 1998).

The proportion of *Ae. vexans* and *An. messeae* in the riverside were the same as in the semi-desert environments, which also was higher than those in the transition zone between these two types of environments. The species of mosquitoes, *Ae. vexans* flourishes in flood environments (O’Malley, 1990). The flood environment in Beiwan accounted for the predominance and high proportion of *Ae. vexans* in the local mosquito community. We noticed that density of *Cq. richiardii*, in traps from one of our sites, decreased with increasing distance from the larval habitat as

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**Table 2** Composition of mosquitoes trapped with the Mosquito Magnet Liberty Plus inside and outside a residential area in Beiwan, China.

<table>
<thead>
<tr>
<th>Species</th>
<th>Within residential areas</th>
<th>Outside residential areas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pi(%)</td>
<td>QTY</td>
</tr>
<tr>
<td><em>Aedes vexans</em></td>
<td>97.83</td>
<td>76,190</td>
</tr>
<tr>
<td><em>Ae. cyprius</em></td>
<td>1.26</td>
<td>983</td>
</tr>
<tr>
<td><em>Anopheles messeae</em></td>
<td>0.56</td>
<td>438</td>
</tr>
<tr>
<td><em>Coquillettida richardi</em></td>
<td>0.01</td>
<td>4</td>
</tr>
<tr>
<td><em>Culex modestus</em></td>
<td>0.16</td>
<td>121</td>
</tr>
<tr>
<td><em>Cx. pipiens pipiens</em></td>
<td>0.18</td>
<td>144</td>
</tr>
</tbody>
</table>

Pi = proportion of each species; QTY = number of mosquitoes

$\chi^2 = 16.486$, $p < 0.01$
observed by other authors (Anno and Takagi et al., 2000 and Leonardo and Rivera et al., 2005). The larvae of *Cq. richiardii* can only be found in reeds from river environments in China (Lu, 1999). This may explain why the proportion of *Cq. richiardii* decreased with an increase in trapping distance from the river.

The proportion of *An. messeae* collected from inside and outside the human residential area were not different. This may be caused by the *Anopheles* mosquitoes. The proportion of *Cx. modestus* and *Cx. pipiens pipiens* were higher in the human residential area than outside, but the proportion of *Ae. cyprius* and *Cq. richiardii* in the outside areas were higher than those in the residential area. The species variation from different trapping sites and environments obtained from our study were similar with other reports from different environments (Ciolpan and Nicolescu et al., 1990). The trapping sites with different amounts of vegetation were significantly influencing the collection of species and amount of floodwater mosquitoes in the study area.

Our study site, located in the transition zone between the river and semi-desert environments, undoubtedly influenced which mosquito species were present and their relative abundance. The proportion of *Ae. cyprius*, *An. messeae*, *Cx. modestus*, and *Cx. pipiens pipiens* at the trapping sites near the river were higher than those at the trapping sites in the center of the human residential area and near semi-desert land. However, in the semi-desert region side, the proportion of *Ae. vexans*, *Ae. cyprius*, *An. messeae*, and *Cq. richiardii* at the sites located in the residential area center were the same as those sites being close to the semi-desert region. There is plentiful vegetation (such as: reeds, weeds,

### Table 3. Proportions of mosquito species at different sample sites from Mosquito Magnet Liberty Plus traps in residential areas close to the wilderness, Beiwan, China.

<table>
<thead>
<tr>
<th>Species</th>
<th>D site</th>
<th>F site</th>
<th>E site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pi (%)</td>
<td>QTY</td>
<td>Pi (%)</td>
</tr>
<tr>
<td><em>Ae. vexans</em></td>
<td>93.99</td>
<td>9,014</td>
<td>98.40</td>
</tr>
<tr>
<td><em>Ae. cyprius</em></td>
<td>1.71</td>
<td>164</td>
<td>1.07</td>
</tr>
<tr>
<td><em>An. messeae</em></td>
<td>2.46</td>
<td>236</td>
<td>0.30</td>
</tr>
<tr>
<td><em>Cq. richiardii</em></td>
<td>0.00</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Cx. modestus</em></td>
<td>0.88</td>
<td>84</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Cx. pipiens pipiens</em></td>
<td>0.96</td>
<td>92</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Note: Pi = proportion of each species; QTY = number of mosquitoes
D site, the riverside site in residential area;
F site, the semi-desert one in residential area;
E site, is located between F site and D site.

χ² = 392.86, p < 0.001

### Table 4. Proportions of mosquito species at different sample sites from Mosquito Magnet Liberty Plus traps located in different wilderness areas outside residential areas, Beiwan, China.

<table>
<thead>
<tr>
<th>Species</th>
<th>A site</th>
<th>C site</th>
<th>B site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pi (%)</td>
<td>QTY</td>
<td>Pi (%)</td>
</tr>
<tr>
<td><em>Ae. vexans</em></td>
<td>96.65</td>
<td>87,880</td>
<td>96.36</td>
</tr>
<tr>
<td><em>Ae. cyprius</em></td>
<td>0.56</td>
<td>512</td>
<td>0.26</td>
</tr>
<tr>
<td><em>An. messeae</em></td>
<td>0.56</td>
<td>512</td>
<td>0.05</td>
</tr>
<tr>
<td><em>Cq. richiardii</em></td>
<td>2.18</td>
<td>1,984</td>
<td>0.05</td>
</tr>
<tr>
<td><em>Cx. modestus</em></td>
<td>0.00</td>
<td>4</td>
<td>0.23</td>
</tr>
<tr>
<td><em>Cx. pipiens pipiens</em></td>
<td>0.04</td>
<td>32</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Note: A site, the riverside outside of residential area;
B site, the semi-desert part site outside residential area;
C site, is located between A site and B site.

χ² = 6201.936, p < 0.001
shrubs, pygmy trees and some arbors.) which might provide good resources and resting sites for adult mosquitoes.

Our results tell us that surveillance of the mosquito density and species composition is important to present good protection for people and livestock. Human-baited bed nets had previously been used for adult mosquito surveillance in this area (Liu and Zhang et al., 2005). Our study found that *Ae. vexans* was the major species in the area (Pi of this species was 99.5%) while *Ae. cyprius* and *An. messeae* were also collected. In addition, the Mosquito Magnet® Traps with octenol collected a great number of floodwater mosquitoes and this method may reduce the nuisance problem in Beiwan where an extremely high density of mosquitoes occurs during the flood seasons. Mosquito Magnet Liberty Plus traps are an efficient surveillance tool for mosquito population and could be considered for use as control measures to reduce the mosquito population in the residential area (Burkett and Lee et al., 2002; Kline, 2006).

V. REFERENCES CITED


ABSTRACT. The host seeking periodicity of *Coquillettidia perturbans* and *Culex erraticus* (two important mosquito bridge vectors of eastern equine encephalitis virus in northern Florida) were determined by using programmable collection bottle rotary traps. Results showed that both species host seeking activity peaked 1 hour after sunset while *Cx. erraticus* continued that level of activity for an additional two hours. On full moon nights, both species displayed peak host seeking activity 1-2 hours longer than that observed during non-full moon nights. High relative humidity significantly increased *Cx. erraticus* abundance in CDC light traps, as well as host seeking activity but not *Cq. perturbans*.

KEY WORDS. *Coquillettidia perturbans*, *Culex erraticus*, host seeking pattern, lunar phase, humidity

I. INTRODUCTION

Knowledge of temporal host seeking patterns of mosquitoes is important for understanding mosquito-host contact, as mosquitoes will only bite hosts that are available during the period of their host seeking activity (Williams 2005). The time of host seeking by mosquitoes delineates periods when females are active and the risk of pathogen transmission greatest for vector species (Reisen et al. 1997). Therefore, determination of the host seeking time (or biting rhythms) exhibited by vector and nuisance mosquito species is essential to decide when adulticides can be most effectively applied. Obviously, climatic factors such as temperature, atmospheric moisture, wind, moonlight, and mosquito physiological age may influence the host seeking behavior of mosquitoes on any given night. Therefore, it is important to have some understanding of the prevailing meteorological conditions and how these conditions modify the behavior of different mosquito species, and consequently the efficacy of controlling those species. During 2008-2009, CDC light traps were placed in four northern Florida counties (Leon, Madison, Holmes and Washington) where positive eastern equine encephalitis (EEEV) equine or sentinel chicken sera were reported to determine what mosquito species were in those areas. *Coquillettidia perturbans* (Walker) and *Culex erraticus* (Dyar and Knab) were the dominant species in all collections and may have been involved in virus transmission. Both species have been incriminated as competent EEEV bridge vectors in North America (Howitt et al. 1949, Boromisa et al. 1987, Cupp et al. 2003, 2004).

McNeel (1932), in an observational study, found that *Cq. perturbans* host seeking activity was greatest at dusk. Snow and Pickard (1957) presented data that indicated *Cq. perturbans* host seeking activity peaked between 1930-2000 h. However, results from these earlier observational studies were not in agreement with a recent study conducted by Bosak et al. (2001). These authors revealed that significantly more *Cq. perturbans* host seeking activity occurred during the night period (between 2200-0100 h) compared with early evening or morning hours. Because of the inconsistencies between the previous studies there is a need to re-examine the host seeking pattern of this species...
in northern Florida especially in areas where EEEV is prevalent.

As stated earlier, *Cx. erraticus* was also one of the more abundant species collected in light traps from EEEV sites and may be an important bridge vector in the area. To this author’s knowledge, host seeking periodicity of this species has not been reported in the scientific literature. Therefore, the aim of this study was to determine the temporal host seeking activity for *Cq. perturbans* and *Cx. erraticus* and identify some of the meteorological factors that may affect timing of this behavior. Obtaining this information will provide data-driven guidance on when adulticides should be most effectively applied.

II. MATERIALS AND METHODS

**Study site.** Field studies were conducted at Leon County’s solid waste management center, 7550 Apalachee Parkway, Tallahassee, Florida (30.25.362 N, 084.08.956 W). This location is one of Leon County Mosquito Control’s permanent sentinel chicken sites that is used to monitor mosquito-borne diseases such as EEEV and West Nile virus in Leon County. This site consisted of a large fresh water swamp (6.1 ha) covered with cattails (*Typha* spp.), arrowhead weeds (*Sagittaria longiloba*), pickerelweed (*Pontederia cordata*), a large forested wood lot, and office buildings. Seropositive EEEV chickens have been reported from this site for the last five years (http://www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/surveillance.html).

**Mosquito collection and processing.** Programmable collection bottle rotator traps (CBR) (Model 1512, John W Hock Company, Gainesville, FL) were used to determine temporal host seeking periodicity of *Cq. perturbans* and *Cx. erraticus*. The CBR is a device that segregates collections into distinct time periods by a programmable timer to which any mechanical mosquito trap, such as a CDC light trap, is attached. In this study, a standard CDC light trap was mounted onto the screen holder located on the top of the CBR unit and secured with thumb screws. A central stainless steel rod extended from underneath the rotator trap and inserted into a tripod lawn sprinkler stand that supported the unit and CDC trap 1.5 meter above the ground. The first collection started at 1943 h (1 hour before sunset) and ended after the collection at 0639h (sunrise). Because each rotator trap only contained 8 bottles, two traps were used to achieve a total of 10 hourly collections. In order to synchronize sunset/sunrise collections, the programmable timer was adjusted accordingly to reflect daily time changes. A separated CDC light trap was set up about 50 m apart from the CBR traps at the edge of the swamp in order to compare species composition between the two collecting methods. CDC and CBR traps were baited with 5 lbs of dry ice contained in a one gallon cooler jug. A small hole was drilled at the bottom of jug so the CO₂ could be delivered to the traps directly. Traps were operated for nine nights during July 1 through August 8, 2009. Mosquito collections were transported to the laboratory, anesthetized, identified to species, and counted. The number of *Cq. perturbans* and *Cx. erraticus* trapped in each hourly bottle collection were summed and an average number of mosquitoes per hour calculated for each species.

**Meteorological data.** On each collection day, temperature, relative humidity, and wind speed were obtained from a www.wunderground.com weather station located about 2 miles from the study site. Moon phase data were obtained from www.moon-connection.com. For this study, the night of a full moon and the night following a full moon were considered as “full moon nights”. (Criterion for this determination was based on the moon phase calendar where the night following a full moon is closer to a full moon than the night before a full moon). A total of four full moon night collections were made in this study, (two nights in July and two nights in August). The remaining sample nights were considered as non-full moon nights.

**Statistical analysis** Mosquito collections were summarized on the basis of the number of females caught per trap per hour. To
determine if temperature, relative humidity, and/or wind speed impacted the host seeking behavior of *Cq. perturbans* and *Cx. erraticus*, one way analysis of variance was used to analyze these relationships using STATISTICA software (StatSoft Inc. 2001) (*P* < 0.05). Hourly mosquito counts were log-transformed [log (n + 1)] to normalize the distribution and relative abundance prior to analysis.

**III. RESULTS**

*Mosquito collection.* Mosquito collection started from July 1 and ended on August 8, 2009. During this 10 week collection period, a total of 9 nightly collections were made. A total of 2,984 female mosquitoes representing 16 species were collected by the CDC light trap, and 1,705 mosquitoes representing 12 species were collected by the CBR traps. *Aedes albopictus* (Skuse), *Ae. inforntus* (Dyar and Knab), *Ae. triseriatus* (Say), *Ae. vexans* (Meigen), *Anopheles crucians* Wiedemann, *Cq. perturbans*, *Cx. erraticus*, *Cx. nigripalpus* Theobald, *Psorophora columbiae* (Dyar and Knab) and *Ps. ciliata* (Fabricius) were collected in both types of traps. The composition of *Cq. perturbans* and *Cx. erraticus* was 40.7%, 48.4% in CDC traps and 37.1%, 57.1% in CBR traps, respectively. Compared with the CBR, more mosquito species (15 species from CDC traps vs. 12 species from CBR traps) and higher numbers of mosquitoes (2,984 female mosquitoes from CDC trap collection vs. 1,705 female mosquitoes from CBR collection) were collected by the CDC traps. In terms of percentage, *Cq. perturbans* collections from CDC traps were slightly greater than CBR traps (40.7% vs. 37.1%); in contrast, a higher percentage of *Cx. erraticus* was collected in CBR traps than CDC trap (57.1% vs. 48.4%), respectively.

*Host seeking periodicity.* Figure 1 shows the temporal host seeking patterns of *Cq. perturbans* and *Cx. erraticus*.

Overall, *Cq. perturbans* host seeking activity started to rise at sunset (SS) then peaked 1 hour after sunset (SS+1), followed by a gradually reduction in activity through the night until one hour before sunrise.

*Culex erraticus* peaked at sunset and sustained a higher level of host seeking activity for an additional two hours (SS+2), followed by a sharp reduction in host seeking activity 3 hr after sunset (SS+3). Activity then gradually slowed down throughout the night. Host seeking activity was lowest 3 hours before sunrise.

*Host seeking periodicity at full-moon and non-full nights.* Figure 2 shows the temporal pattern of host-seeking activity for *Cq. perturbans* and *Cx. erraticus* as influenced by lu-
nunar phases. Both species delayed their peak host seeking activity at least 1 hour on full moon nights than non-full moon nights accompanied by an increase in the number of mosquitoes trapped during this extended activity period. In terms of $Cq.\ perturbans$, its peak activity was one hour later at full moon nights ($SS+2$) than that of non-full moon nights ($SS+1$). The number of mosquitoes collected at $SS$ time and $SS+1$ were not much different (Figure 2), however, the numbers collected at $SS+3$, $SS+4$, $SS+5$ and $SS+6$ were much higher (no statistical significantly differences) at the full-moon nights than that of the non-full moon nights. Furthermore, $Cq.\ perturbans$ extended its activity by two hours on full-moon nights ($SS$ to $SS+3$) compared with non-full moon nights (i.e. $SS$ to $SS+1$).

$Culex\ erraticus$, on the other hand, exhibited a two hour delay in peak host seeking activity on full-moon nights ($SS+2$) compared with non-full moon nights ($SS$) (Figure 2). Significantly more mosquitoes ($P<0.05$) were trapped at $SS+1$, $SS+2$ and $SS+4$ on full moon nights than non-full moon nights.

Host seeking periodicity under different meteorological conditions. Host seeking activity of $Cq.\ perturbans$ was not significantly influenced by changes of temperature, relative humidity, or wind speed ($P<0.362;\ P<0.463;\ P<0.088$; respectively). Temperature and wind speed did not significantly effect $Cx.\ erraticus$ ($P<0.48;\ P<0.263$; respectively). However, significant ($P<0.01$) higher numbers of $Cx.\ erraticus$ were trapped at higher humidity (85-95%) than low humidity and indicated that this meteorological factor altered $Cx.\ erraticus$ host seeking behavior.

IV. DISCUSSION

Information regarding the precise timing of host seeking by $Cq.\ perturbans$ and $Cx.\ erraticus$ is essential for the optimal timing of adulticide missions. This study clearly indicated that $Cq.\ perturbans$ host seeking activity started at sunset and sustained a peak level of activity for at least two hours. This is in contrast to that reported by Snow and Pickard (1957) where peak host seeking occurred between 1930-2000 h and Bosak et al. (1987) from 2200 to 0100 h. It is likely that mosquitoes react to light intensity crepuscular changes (sunset, sunrise) to initiate their flight and host seeking rather than actual “clock time” because the periods of sunrise/sunset change over the seasons. Moreover, temporal host seeking by $Cq.\ perturbans$ may vary in different geographic regions and the use of crepuscular light intensity changes.
may be a more appropriate indicator of host seeking flight behavior.

Although *Cx. erraticus* dispersal behavior (Estep et al. 2010), host feeding preference (Oliveira et al. 2011; Burkett-Candena et al. 2012; Mendenhall et al. 2012) and general bionomics (Robertson et al. 1993) have been extensively studied, temporal host feeding periodicity has not been reported. This is the first report to describe accurately the timing of host seeking behavior of *Cx. erraticus* in northern Florida, as well as the influence of meteorological conditions, such as moon phase and relative humidity, on host seeking behavior.

*Coquillettidia perturbans* and *Cx. erraticus* are considered crepuscular-nocturnal species; however, their host seeking patterns have similarity and differences. For example, both species consistently commenced host seeking at sunset, either continued or slightly increased its host seeking activity for the next two hours. However, *Cq. perturbans* reduced its host seeking activity gradually at SS+2, whereas, *Cx. erraticus* host seeking activity abruptly dropped at SS+2.

Many mosquito species have their greatest period of flight activity during twilight (Mitchell, 1982; Nelson and Spadoni, 1972; Reisen et al. 1983). Moonlight can simulate twilight conditions by increasing nocturnal illumination. This study also illustrates that *Cq. perturbans* and *Cx. erraticus* significantly their increased activity and an extended activity period under the full moon phase (Figure 2), which is consistent with early studies. Antonipulle et al. (1958) studied the host seeking behavior of *Mansonia uniformis* (Theobald) using cattle-baited traps. Analysis of the catches by hour showed that during each lunar phase, the highest approach rates occurred during the periods of full moonlight. Ribbands (1946) also found that the number of *Anopheles funestus* Giles entered man-baited huts was significantly correlated with the times of twilight and of moonlight. Enter rates were low during moonless periods of the night and highest at the full moon period. However, exactly the opposite results were reported by Chadee and Tikasingh (1989). Their studies showed that over 70% mosquito *Culex caudelli* Dyar and Knab captured in mouse-baited traps were collected between 2200 and 0400 h. They compared the collections made during the four lunar quarters and found out that neither the extent of host seeking activity nor its timing was affected by moon phase. The impact of lunar phase on the host seeking patterns of mosquitoes may vary by species, physiological age, and meteorological conditions (Provost 1959).

It is important to understand ambient meteorological conditions as they affect the behavior of various mosquito species. Temperature, relative humidity, and wind speed affect mosquito behavioral periodicities as permissive factors. This study showed that there was no correlation between host seeking periodicity and the meteorological parameters recorded during collections for *Cq. perturbans*. However, relative humidity had a significantly \((P < 0.01)\) positive impact on *Cx. erraticus* host seeking patterns. A statistically significant increase in the number of *Cx. erraticus* collected during periods of high relative humidity (85-95\%) was reported in this study. Day and Curtis (1989) reported a significant positive correlation between blood feeding by *Cx. nigripalpus* and relative humidity. Dow and Gerrish (1970) also reported a positive correlation between host seeking of *Cx. nigripalpus* in baited traps and humidity with a 4.8\% increase in mosquito catches for each 1\% increase of relative humidity. Evidently, the meteorological factors such as humidity had influence on *Cx. erraticus* host seeking behavior but not *Cq. perturbans*. The impact was more species-specific, as in the case of *Cx. erraticus* in this study.

The precise timing of an adulticide spray application designed to intersect with maximum adult mosquito flight behavior is critical for effective mosquito control. This study reveals the accurate timing of the crepuscular/nocturnal flight patterns of *Cq. perturbans* and *Cx. erraticus*, two potential EEEV bridge vectors in North Florida. Results from this study could be providing the optimal time for controlling the two species of mosquitoes and reducing the risk of EEE virus transmission in North Florida.
V. ACKNOWLEDGEMENTS

The author would like to thank Leon County Mosquito Control for helping to identify mosquito trap locations and agreeing to use their sentinel chicken sites for this study.

VI. REFERENCES CITED


FIELD EVALUATION OF THREE COMMERCIAL MOSQUITO TRAPS AND FIVE ATTRACTANTS IN NORTHEASTERN FLORIDA

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ABSTRACT. Three commercial mosquito traps and five attractants were evaluated in northeastern Florida. The combinations of traps and attractants were BG Sentinel (BGS) + BG Lure, Mosquito Magnet X (MMX) + CO2, Mos-Hole + naphtha, MMX + octenol, Mos-Hole + BG Lure, BGS + octenol, and BGS + Lurex3. The MMX trap paired with CO2 collected the greatest number of mosquitoes and the MMX trap paired with octenol trapped the least amount compared with the rest of the trap/attractant combinations.

Key Words. BG Sentinel trap, Mos-Hole trap, Mosquito Magnet X trap, BG lure, octenol

I. INTRODUCTION

Mosquitoes are highly dependent on their olfactory capabilities to host seek (Anthony and Rospars 2004). Semiochemicals (e.g. octenol and CO2) and physical characteristics (such as convection currents and warmth) can signal the presence of a host. Historically, mosquito traps have been baited with a variety of attractant substances that take advantage of the host seeking instinct by mimicking a blood meal source or chemicals exuded by animal hosts. Generally, from the perspective of the Anastasia Mosquito Control District (AMCD), there are no traps or attractants that are universally attractive to all 43 species of mosquitoes found within its jurisdiction.

A number of studies have reported that Mosquito Magnet® X traps (MMX) with CO2 and other attractants (including lactic acid, octenol, ammonia, or fatty acids) collect a variety of mosquito species (Kline et al. 1991, 2006, Ritchie et al. 2006, Farajollahi et al. 2009, Hoel et al. 2009, Gilek et al. 2011, Xue and Smith 2013). Recently, Xue et al. (2015) found the novel Mos-Hole™ trap, baited with naphtha, was an effective tool for surveillance of anthropophilic mosquitoes when compared with BioGents Sentinel™ (BGS) and MMX traps. In this paper we report on additional field evaluations we conducted regarding the attractiveness of BGS, MMX, and Mos-Hole traps baited with either CO2, naphtha, octenol, lactic acid, or a commercial BG Lure™ to collect adult mosquitoes in St. Johns County, Florida.

II. MATERIALS AND METHODS

Field trials were conducted in St. Johns County during June and July 2013. The location of the study site was off of County Road 214 (29.87.13N, 81.36.98W). This low-lying hardwood habitat contained several hundred used tires (16 m W × 133 m L × 4 m H) surrounded by agricultural land with shallow ditches. BGS (white cover version) (BioGents, Regensburg, Germany), Mos-Hole (Lnd E-TND Co. Ltd., Hanam City, Gyeongi-Do, Republic of Korea), and MMX (Woodstream Corporation, Lititz, PA) traps were used in this study and powered by 12v batteries. The Mos-Hole trap was a dark blue unit placed on the ground surface similar to how BGS traps are positioned.

The following five attractants were tested: BG Lure (ammonia, lactic acid, and fatty acids, BioGents, Regensburg, Germany), liquid naphtha (Lnd E-TND Co. Ltd., Hanam
City, Gyeongi-Do, Republic of Korea), CO$_2$ (dry ice block from local supplier), octenol (BioSensory, Willimantic, Connecticut) and Lurex3 (ammonia and lactic acid, Woodstream Corporation, Litiz, PA). A total of seven trap/attractant combinations were evaluated (Table 1). Liquid naphtha is a flammable liquid hydrocarbon that when combined with air, produces heat and CO$_2$ that was specifically designed for the Mos-Hole trap (Xue et al. 2015). The liquid was contained in a polyethylene bottle and placed on the bottom surface inside the trap. Also a mesh pouch was sewn into the Mos-Hole trap to mimic the pouch of the BGS, so that both traps were able to contain attractants. The MMX trap was suspended from a shepherd’s hook so that the trap opening was approximately 0.5m from the ground surface. Carbon dioxide was dispensed to this trap from a pressurized gas cylinder through 5 mm silicon tubing and controlled using a flow-meter. BG Sentinel and MMX traps plus naphtha and MMX plus Lurex3 were not evaluated in this study, because they had been evaluated earlier by Xue et al. (2015). Traps were set up in the shade in a linear fashion, 10 m apart, and >15m from the closest tire pile. Traps were rotated and rebaited daily after collection. After each 24-hour trapping period, the labeled collection bags were transported to the AMCD laboratory, frozen to -20° C, and identified to species using the taxonomic keys of Darsie and Ward (2004).

Statistical analysis. Mean mosquito abundance from each trap/lure combination was separately pooled by genera (Aedes, Culex, and Psorophora) and subjected to one-way ANOVA using JMP® ver. 11.1 software (SAS Institute, Inc. 2013) after log transformation of trap count data. Differences were considered significant at $P \leq 0.05$. A Tukey-Kramer HSD multiple mean test was used to establish genera differences within trap/lure combinations.

### III. RESULTS AND DISCUSSION

A total of 1,207 mosquitoes from 15 species were collected during the study period representing 8 genera. Overall mosquito composition and abundance in collections were as follows: Aedes albopictus (Skuse) (27.9%), Culex nigripalpus Theobald (26.6%), Ae. infirmatus (Dyar and Knab) (26.5%), Culiseta melanura (Coquillett) (4.0%), Psorophora ferox (von Humboldt) (3.2%), Ps. columbiae (Dyar and Knab) (3.0%), Ae. atlanticus (Dyar and Knab) (1.8%), Anopheles quadrimaculatus Say (1.6%), An. crucians Wiedemann (1.6%), Ae. triseriatus (Say) (0.8%) Ae. fulvus pallens (Ross) (0.2%), Ps. howardi Coquillett (0.1%), Toxorhynchites rutilus (Coquillett) (0.1%) and Cx. quinquefasciatus Say (0.1%). Of the total mosquitoes collected 0.7% were unable to be identified due to damage.

There was no statistical difference when traps were compared with total mosquitoes collected ($F = 2.984, P = 0.055$) as well as attractants and mosquito totals ($F = 1.778, P = 0.138$). Overall, there was a significant difference in mosquito abundance between the seven trap/attractant combinations ($F = 6.240, P = 0.004$). The MMX trap paired with CO$_2$ collected the greatest number of mosquitoes followed by BGS+Lurex3, BGS+BG Lure, Mos-Hole+naphtha, Mos-Hole+BG

<table>
<thead>
<tr>
<th>Trap</th>
<th>Culex</th>
<th>Aedes</th>
<th>Psorophora</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMX + CO$_2$</td>
<td>84.7a</td>
<td>39.7b</td>
<td>7.3c</td>
</tr>
<tr>
<td>BGS + Lurex3</td>
<td>4.0c</td>
<td>23.0bc</td>
<td>6.0c</td>
</tr>
<tr>
<td>BGS + octenol</td>
<td>2.0bc</td>
<td>12.0c</td>
<td>1.5c</td>
</tr>
<tr>
<td>BG + BG Lure</td>
<td>2.3c</td>
<td>6.6c</td>
<td>2.0c</td>
</tr>
<tr>
<td>Mos-Hole + BG Lure</td>
<td>2.0c</td>
<td>11.7c</td>
<td>2.0c</td>
</tr>
<tr>
<td>Mos-Hole + naphtha</td>
<td>8.5c</td>
<td>10.9c</td>
<td>1.5c</td>
</tr>
<tr>
<td>MMX + octenol</td>
<td>1.0c</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Means followed by the same letters in a row are not significantly different (*P* >0.05).*
Lure, BGS+octenol, while the MMX trap paired with octenol trapped the least amount of mosquitoes.

No significant difference existed between each of the 15 mosquito species trapped by the 7 trap/attractant combinations. However, there were differences ($F = 3.589, P = 0.0001$) between abundance of the three genera within trap/attractant combination (Table 1). MMX traps plus CO$_2$ collected significantly more Aedes and Culex mosquitoes than Psorophora. However, trap abundance for the rest of the trap/attractant combinations were no different between genera.

Previously, Xue et al. (2015) found that Mos-Hole traps baited with naphtha were an effective tool for surveillance and control for anthropophilic mosquitoes compared with BGS+BG Lure and MMX traps baited with Lurex3. That study did not find differences in the number of individuals, per genera, from BGS traps baited with naphtha. Xue et al. (2015) also reported that Mos-Hole traps baited with BG Lure collected greater numbers of Aedes mosquitoes than BGS traps baited with naphtha. We noticed the same trend for Mos-Hole traps baited with either BG Lure or naphtha in our study (Table 1).

We also found that the MMX trap plus octenol was least effective. This contradicted the results from Xue et al (2010) who reported that the same trap/attractant combination collected more floodwater mosquitoes than similar traps baited with Lurex3 and a CO$_2$ sachet. Xue et al. (2010) also found Lurex3 and octenol were effective attractants when combined with MMX traps or the Mosquito Magnet® Pro, in order “to improve the collection and possibly the management of floodwater mosquitoes”. Although the BGS trap plus Lurex3, or BG Lure, did not collect as many total mosquitoes (as MMX plus CO$_2$) they captured the second highest number of Psorophora. Our data suggests that the BGS trap/Lurex3 combination targeted floodwater species more than expected because the attractant sachet (which contained components of human odor) should have drawn more anthropophilic mosquitoes to the trap.

Out of all seven different trap and attractant combinations tested, the MMX trap paired with CO$_2$ was the overall best combination. Hoel et al. (2007) reported similar results where un-baited Mosquito Magnet Pro traps caught the largest number of floodwater species. Moreover, these authors also found that their traps, when baited with octenol and lactic acid, actually decreased collection abundance. In studies by Xue et al. (2008) and Zhu et al. (2014) dry ice baited MMX traps were very effective for collecting floodwater mosquitoes from multiple field sites.

MMX traps remain effective tools for mosquito surveillance because the units intake entrance, while positioned at the bottom, takes advantage of the natural tendency of mosquitoes to fly up. Moreover, MMX traps are ideal for research purposes when mosquitoes need to remain alive during collection. The trap’s collection chamber is able to hold a water-soaked cotton ball for moisture and nutritional requirements if this is required by the trapped mosquitoes. The BGS and Mos-Hole traps have fairly small mesh collection bags. Mosquito specimens in these traps tend to be easily desiccated and damaged by the air velocity of their intake fans.

In summary, continuing studies are warranted that evaluate the effectiveness of traps without attractive substances and/or combining additional attractants in order to determine additive or synergistic effects on trap abundance and species composition. Comparative studies should consider evaluation with traps that are currently considered the gold standard for mosquito surveillance in order to increase the effectiveness of operational programs.

IV. ACKNOWLEDGMENTS

The authors are grateful to Claudia Davidson, Tanjim Hossain, Richard Weaver, and Kay Gaines for their technical help. This is a research report only and does not endorse any of the commercial products involved or mentioned in this report.

IV. REFERENCES CITED

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FIELD EVALUATION OF MOSQUIRON 0.12CRD AGAINST 
CULEX QUINQUEFASCIATUS IN STORM DRAINS,
DOWNTOWN ST. AUGUSTINE, FLORIDA

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ABSTRACT. A controlled release formulation of the chitin synthesis inhibitor novaluron (Mosquiron 0.12 CRD) was evaluated in downtown St. Augustine, FL storm drains for 8 weeks. Results suggested that the treatments provided effective control of larval Culex quinquefasciatus in storm drains that were cleaned of debris prior to treatment. Synchronizing treatment with municipalities’ drain debris cleaning schedules may improve the effectiveness of this formulation against mosquito larvae.

Key Words. Culex quinquefasciatus, novaluron, insect growth regulator, storm drain

I. INTRODUCTION

In Florida, the number of suburban and urban storm drains has been steadily increasing due to continued growth associated with residential and infrastructural development. As the number of storm drains have increased, their water and debris holding capacity have also increased as a result of recent National Pollutant Discharge Elimination System (NPDES) regulations (Metzger 2004). As older infrastructure is replaced with NPDES compliant ones, a subsequent increase in potential larval production sites may be inevitable (Su et al. 2003a). An increase of these new storm drains in close proximity to humans increases the challenges faced by the Anastasia Mosquito Control District (AMCD).

West Nile virus vectors, like Culex quinquefasciatus Say, thrive in storm drains and will readily feed on humans (Moulis et al. 2013). Treatment of individual storm drains by mosquito control district personnel can be laborious, time consuming, and costly. Many controlled release larvicide formulations are expensive. Some sink to the bottom and are buried in sediments, while others may be washed away during heavy storm and rainfall events. Anastasia Mosquito Control District currently uses Bacillus thuringiensis israelensis (Bti) liquid in downtown St. Augustine storm drains to achieve successful control. However, this formulation has a limited treatment window where application must occur before fourth instar development because feeding slows down as they approach later instars. The other challenges of Bti formulations include short residual life and the adverse impact that organic matter, in the drain, has on product efficacy.

Past treatment of storm drain habitats by AMCD in downtown St. Augustine has focused on thermal fogging and toxic bait stations to control adult mosquitoes (Müller et al. 2010, Xue et al. 2012, Xue et al. 2013). Controlling larval stages in storm drains before they become adults has advantages over adult control. The ideal profile of a larval control product is that it would be reasonable in cost, provide long residual efficacy, and remain in the application location through significant rainfall events. With an increase in storm drains with higher capacities, new larvicide products that provide controlled release of the active ingredient are critically needed. Therefore, we report here on the evaluation of a novel controlled release larvicide formulation, Mosquiron® 0.12 CRD, (novaluron 0.12%AI) against mosquito larvae in downtown storm drains in St. Augustine, Florida.
Since 2003, various formulations of novaluron have been evaluated against *Culex* species in laboratory and field studies (Su et al. 2003b). Interestingly, application rates that achieved <80% control appear to be species dependent (Arredondo-Jimenez and Valdez-Delgado, 2006). The mode of action of novaluron is that of a chitin synthesis inhibitor within the class of insect growth regulators. Early studies found lower concentrations of novaluron primarily resulted in inhibition emergence of adults, while higher levels primarily produced larval mortality (Su et al. 2003b, 2014). The World Health Organization recommended novaluron for mosquito larval control (WHO 2006) and also approved it for use in drinking water.

Mosquiron CRDs have been evaluated with success in multiple water bodies, but only one earlier study has explored the laboratory activity and field efficacy of this product in storm drains or catch basins in North America (Su et al. 2014). The product label does not allow treatment of water directly connected to natural water bodies but does permit the treatment of catch basins or storm drains. Residual activity of novaluron depends on water characteristics, such as volume and presence of organic debris. Mosquiron CRDs are designed to deliver waxy particles containing novaluron in a short period of time, while the active ingredient is released from the particle and adheres to the surfaces of the storm drain and sediments for continual release.

**II. MATERIALS AND METHODS**

Twelve storm drains in downtown St. Augustine were selected for this study that contained approximately six inches (15.2 cm) of standing water containing mosquito larvae previously determined by dipper samples. All drains were similar in size and structure. Eight drains were used in the study. Four drains were utilized for novaluron treatment. The remaining drains were untreated controls located at a minimum of 30 meters from the first treatment site. No untreated control sites were connected to the treatment storm water drainage lines, inflow, or outflow, according to the storm water system map provided by the City of St. Augustine. No Bti treatments were made in the study sites by AMCD or adjacent storm drains a week prior to, or during, the study period.

One CRD was placed in each of the treatment storm drains according to the label. The CRDs were cylindrical in shape, 5-10 cm length by 1-2 cm diameter, and weighed 13.8 g/each. A 200 to 350 ml water sample was removed weekly from each drain using a white plastic handheld-dipper (12.7 cm diameter 5.1 cm deep, Clarke ABC Dipper with Telescoping Handle, St. Charles, IL). Samples from each drain were individually placed into clear plastic mosquito emergence containers (Bioquip, Rancho Dominguez, CA), labeled by site location and date, then transported to the AMCD insectary. If non-target organisms were present in samples they were placed in separate containers, with their sample water, and any adverse effects recorded during the experiment.

Mortality in aquatic stages was recorded every 48 hours and adult mosquito inhibition weekly through 14 days. If no larvae were present in storm drain samples then ten laboratory-reared *Cx. quinquefasciatus* early third instar larvae were added and recorded as above. Larvae in each emergence container were maintained in AMCD’s insectary (12L:12D, 26.6°C, >70% RH) and weekly fed approximately 0.03 g of ground dog biscuits.

At post treatment week 4, retreatment was necessary due to heavy rains that resulted in a considerable decrease in novaluron efficacy. In this trial 2, each of the eight treatment drains (control included) were treated. Four additional untreated drains were used as controls. During trials 1 and 2 weekly precipitation, water level, and vegetation/debris contents were recorded for each drain. The City of St. Augustine staff also collected salinity, dissolved oxygen, and pH in the drains for the study from April to September 2014.

*Statistical analysis* ANOVA and a subsequent Student’s *t* test were run with JMP® statistical software (SAS Institute Inc. 2013).
III. RESULTS AND DISCUSSION

Average water temperature in the storm drains was 23.2° C; salinity averaged 12.4 parts per thousand; dissolved oxygen averaged 0.41 mg/l; and the average pH was 6.8. Salinity was the only measure that varied. One week after treatment, novaluron produced an average of 73% adult inhibition of *Cx. quinquefasciatus* (Figure 1). Efficacy was affected by rainfall after week 1, which appeared to have washed the CRD components out of the drain before they had time to adhere to the sides of the drains. Adult emergence was reduced to 23 and 33% for weeks 2 and 3, respectively, but rose again to 78% at week 4. At that time, efficacy was unacceptable so drains were retreated on week 4. Retreatment on week 4 provided 98% emergence inhibition in week 5 samples and remained between 68 and 84% for the duration of the study.

Overall, there was a statistically significant difference in treatment versus control in adult emergence inhibition (*P* = 0.002). There was no significant difference between treatments and controls in trial one (*P* = 0.05), while a statistically significant difference was found in trial two between treatment and controls (*P* = 0.004). Retreatment on week 4 appeared to have boosted the emergence inhibition despite the later rain events (Figure 1). When larvae were found in higher numbers during sampling, emergence inhibition was significantly greater (*P* = 0.03). Similarly, when we focused on trial two, lower numbers of larvae in the drain samples exhibited higher emergence inhibition and was statistically significant (*P* = 0.02).

The City provided a storm drain cleaning schedule that allowed us to track which drains had been cleaned during our study. For drains cleaned most recently, the mean emergence inhibition was higher than those not cleaned recently or upon initiation of the study (*P* = 0.04). Lower levels of vegetation and debris in drains resulted in statistically significant higher levels of emergence inhibition (*P* = 0.04). Wax from the novaluron treatment was observed in multiple treated drains post treatment on the sides of the structures and on debris (especially mulch chunks). When debris was minimal, or non-existent, the wax appeared to adhere more to the sides of the drains thereby increasing residual efficacy of the formulation. The challenges to the study were the significant rain events.

We did not observe differences in non-target mortality between treatments and controls. Similarly, Su et al. (2003b) found

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**Figure 1.** Weekly mean percent adult emergence inhibition of *Culex quinquefasciatus* after treatment of storm drains by Mosquiron 0.12CRD (novaluron) at wks 0 and 4 and accompanying precipitation levels during the study.
the novaluron 10% EC formula safe for non-targets in outdoor mesocosms at the application rate used for mosquito control. The majority of non-targets in our study were copepods and our observations were also confirmed by Su et al. (2003b) and Arredondo-Jimenez and Valdez-Delgado (2006) that these crustaceans are not adversely affected by novaluron. Although we did not collect larvivorous fish from the storm drains, Paiva et al. (2014) found 2% novaluron did not affect these predators. In addition, Tawatsin et al. (2007) did not observe negative impacts on fish when treating for Cx. quinquefasciatus with 10% EC novaluron in polluted water.

Our average emergence inhibition of Cx. quinquefasciatus with the CRD formulation of novaluron was not as high as reported by Arredondo-Jimenez and Valdez-Delgado (2006) and Su et al. (2003b) who used a 10% EC formulation. Other studies, such as Jambulingam et al. (2009), involving different formulations of novaluron found high emergence inhibition for Cx. quinquefasciatus in wells. Additionally, Mulla et al (2003) found that 10% EC and technical material had long term activity in controlling Ae. aegypti in storage containers. However, our second treatment with the CRD formulation produced greater inhibition and may have benefited from the previous application.

In summary, Mosquiron CRDs can be an effective control agent against the production of adult mosquitoes in recently cleaned storm drains over the course of multiple weeks. As the City of St. Augustine continues to follow Best Management Practices for urban storm water runoff, working in conjunction with AMCD in reducing the abundance of Cx. quinquefasciatus in these systems will benefit the general public, at large, by reducing the nuisance and risk of disease transmission from this species.

IV. ACKNOWLEDGEMENTS

This is a research report only, AMCD does not endorse any commercial products mentioned. We would like to thank John Regan, Todd Grant, Reuben Franklin, Glabra Skipp, and Jeanne Moeller for their invaluable support and collaboration. Also, we thank Barry Tyler (Pest Alto Environmental Products Inc., Guelph, ON, Canada) for providing partial funding and product samples. The assistance of Tom Downey, Ken Daniels, Emily Thomson, and Rick Stockley in the field during this evaluation was appreciated.

V. REFERENCES CITED


SAS Institute, Inc. 2013. JMP, Version 11.1 Cary, NC.


ABSTRACT. A two-year study was conducted to evaluate the effectiveness of Mosquito Magnet® Liberty Plus (MMLP) traps to reduce mosquito and biting midge abundance in a St. Augustine Beach, FL golf community bordered by salt marsh habitat. These traps were placed in residential backyards on the fringe of the salt marsh for the purpose of reducing the number of mosquitoes and biting midges entering the central residential area. These traps collected a total of 35,265 mosquitoes from 9 genera where *Aedes taeniorhynchus* (28%) and *Anopheles crucians* (27%) were the two major species. Mosquito Magnet X (MMX) traps were used to monitor mosquito and biting midge population abundance in the residential backyards. These traps collected 8,998 mosquitoes from 5 genera with *Culex nigripalpus* (75%) as the major species. More than one million biting midges were collected from both residential areas with *Culicoides furens* (95%) being the dominant species. More than one million biting midges were collected from both residential areas with *Culicoides furens* (95%) being the dominant species. Mosquito and biting midge abundance from the MMX traps in residential backyards were significantly reduced by 59 and 23%, respectively, compared with trap abundance from the residential areas with MMLP traps in the adjacent salt marsh. However, mosquito landing rates were not significantly reduced in the residential areas.

Key Words. *Aedes taeniorhynchus*, *Anopheles crucians*, *Culex nigripalpus*, *Culicoides furens*, Mosquito Magnet Liberty Plus trap, Mosquito Magnet X traps, biting midges

I. INTRODUCTION

Mosquito traps combined with attractants have been evaluated by several researchers and remain the standard for adult mosquito surveillance (Allen et al. 2009; Chaves et al. 2014; Jackson et al. 2012). Recently, many commercial mosquito traps have integrated attractants into their units for the express purpose of providing control of adult mosquitoes (Kline 2006, Xue et al. 2008). These types of traps have attracted homeowner attention due to safety concerns regarding pesticide-based mosquito control technology. A variety of traps and lures that emit host cues have been marketed for the general public. A prime example is the Mosquito Magnet® mosquito trap series (Woodstream Corp., Lititz, PA) that has been marketed worldwide to consumers as a convenient, effective method to control adult mosquitoes outdoors.

Mosquito Magnet traps have been evaluated against a variety of nuisance mosquitoes (Henderson et al. 2006; Jackson et al. 2012; Kitau et al. 2010; Smith et al. 2010) and biting midges (Cilek and Hallmon 2005; Lloyd et al. 2008). However, these traps have not been evaluated for their effectiveness at re-
ducing major mosquito and biting midge populations in large residential/recreation-
al areas (such as golf club communities) with surrounding salt marsh habitat.

The purposes of this study were to: (1) determine species composition of mosqui-
toes and biting midges in the salt marsh habitat and central residential area in a large
golf course community, (2) evaluate whether Mosquito Magnet Liberty Plus traps can
reduce population abundance of the major mosquito and biting midge species encoun-
tered in the residential areas, compared with the salt marsh habitat, and (3) determine
whether traps can reduce human landing rate counts of mosquitoes in the residential
area.

II. MATERIALS AND METHODS
This study was conducted from July to
October, 2004-2005, at the Marsh Creek
Golf Course on Anastasia Island, St. Augus-
tine Beach, Florida. This golf course covers
9,333 acres that includes a residential com-

munity with about 400 homes. The habitat
surrounding the golf course is primarily
salt marsh with low-lying grassy areas that
will retain rainwater for days at a time. The
central residential area and residential back-
yards adjacent to the salt marsh were each
divided into 2 sections. In one area, 16 Mos-
quitos Magnet Liberty Plus (MMLP) traps
(American Biophysics Corporation), baited
with octenol, were placed at 500 m intervals
in central residential backyards. The same
number of traps, and set up, were placed in
the backyard of homes adjacent to the salt
marsh. Residences with MMLP traps will be
referred to as “treatments” and were oper-
ated from Monday through Thursday for
4 nights per week. Controls consisted of
homes in each residential area without traps.
Treatment and control areas were switched
back and forth weekly, within each residen-
tial area, during the 6 wk study. In order to
obtain additional information of mosquito
abundance in the central residential section
with MMLP traps, landing rate counts (LRC)
were conducted (2 m away from each trap)
by a volunteer who recorded the number of
mosquitoes landing on both forearms for 3
minutes at the time of MMLP collection.

In order to monitor temporal mosquito
and biting midge abundance in the treat-
ment and control sites, a MMX trap was
placed in each of two backyards in all areas.
Traps were suspended by a shepherd’s hook
and baited with dry ice (Xue et al. 2008).
Distance between MMX traps in each area
was about 1.5 km while the distance between
MMX traps and MMLP traps ranged from
1-3 km. MMX traps were turned on a Thurs-
day and collected 24 h later on Friday. Mos-
quitos and biting midge collections from all
MMLP and MMX traps were transported to
the laboratory for species identification and
counting under a 10-40X-zoom dissection
microscope.

Statistical analysis. All statistical analyses
were performed using SAS (SAS Institute
2001). Prior to analyses, mean trap counts
were log (x+1) transformed then subjected
to PROC MIXED with a repeated measures
analysis of variance (ANOVA). A χ² test was
performed on mosquito collections to de-
termine if differences existed between treat-
ment and control areas (P<0.05).

III. RESULTS
A total of 35,265 mosquitoes represent-
ing 27 species from 9 genera were collected
from all MMLP traps from backyard resi-
dences adjacent to the salt marsh. The spe-
cies composition is presented in Table 1. The
major species of mosquitoes in this area was
Aedes taeniorhynchus (Wiedemann) (28%),
Anopheles crucians Wiedemann (27%), Culex
nigripalpus Theobald (12%), Psorophora co-
lumbiae (Dyar and Knab) (9%), Aedes sollici-
tans (Walker) (6%), and Ae. infirmatus (Dyar
and Knab) (5%). Total abundance from all
MMLP traps in the central residential sec-
tion totaled 8,998 mosquitoes and contained
10 species from 6 genera. The dominant spe-
cies in these traps was Cx. nigripalpus (75%)
(Table 2). Collections of all MMX traps from
residential areas adjacent to the salt marsh
(with MMLP traps) totaled 27,072 mosqui-
toes and 495,780 biting midges (95% Cul-
icides furans and 5% C. mississippiensis) while
45,579 adult mosquitoes and 2,195,533 biting midges were collected from MMX traps in the same area without MMLP traps.

Mosquito and biting midge collections from MMX traps in MMLP residential backyards adjacent to salt marshes were significantly reduced by 59 and 23%, respectively, compared with trap abundance in similar areas without MMLPs. This trend continued in the central residential area where MMLP traps provided about a 63% reduction in the number of mosquitoes in this area compared with the same area without traps (Table 2). Unfortunately, mosquito LRCs in central residential and salt marsh areas with MMLP traps were not significantly different from LRCs in areas without traps (Table 3).

IV. DISCUSSION

Our study found that Mosquito Magnet Liberty Plus traps significantly reduced mosquito and biting midge populations in the central residential area of the Marsh Creek Golf Course. Similar population reduction has been observed by other in trap out studies where Mosquito Magnets have been evaluated (Cilek and Hallmon 2005, Kline 2002, 2007, Collier et al. 2006, Qualls and Mullen 2007, Lloyd et al. 2008, Li et al. 2010). Unfortunately, Mosquito Magnet Liberty traps placed in residential backyards adjacent to the salt marsh or within the central residential of the golf course did not result in a significant reduction of landing rate counts, compared with areas without MMLPs. It is possible that the sites where landing rates were conducted were too close (2 meters) to the MMLP sites where more mosquitoes may have been present. However, MMLPs did collect a large number of mosquitoes that had significantly reduced the number of mosquitoes in those areas.

The deployment of a linear array of Mosquito Magnet traps in residential backyards that are adjacent to salt marsh areas may be considered to be an environmentally friendly method to reduce the nuisance mosquito and biting midge problems, especially in sensitive habitats. This control method could be easily adopted and accepted by the residents who live by salt marshes.

V. ACKNOWLEDGEMENTS

The authors wish to thank Alex Santoro and James Wynn for their assistance on the project. We also thank A. Grant and K. McKenzie from the former American Biophysics Corporation for providing the Mosquito Magnet Liberty Plus and Mosquito Magnet-X traps. We also the May Management Company and many residents in Marsh Creek to allow us to use their properties during the study. This is a research report only and

<table>
<thead>
<tr>
<th>Species</th>
<th>Salt marsh side (%)</th>
<th>Central residential area (%)</th>
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</thead>
<tbody>
<tr>
<td>Aedes albopictus</td>
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</tr>
<tr>
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<td>2</td>
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</tr>
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<td>0</td>
</tr>
<tr>
<td>Ae. infirmatus</td>
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</tr>
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<td>0</td>
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<tr>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Ae. vexans</td>
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χ² = 4260.81, df = 26, P<0.01
mention of specific products does not indicate endorsement by the AMCD. Volunteers gave informed consent prior to participation in the study and this research study was conducted according to protocol number AMCD 10-13-2005 as approved by the Anastasia Mosquito Control Board of Commissioners for use of human subjects in operational projects.

VI. REFERENCES CITED


SUBLETHAL DOSES OF AN ATTRACTIVE TOXIC SUGAR BAIT MIXED WITH THE INSECT GROWTH REGULATOR PYRIPROXYFEN DID NOT EFFECT SURVIVAL OR FECUNDITY OF Aedes aegypti

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ABSTRACT. Addition of the insect growth regulator, pyriproxyfen (0.5%), into an attractive toxic sugar bait solution containing a sublethal dose of eugenol (0.0125%) or boric acid (0.1%) had no significant effect on the survival or fecundity of adult Aedes aegypti compared with sugar baits without these active ingredients.

Key Words. Aedes aegypti, pyriproxyfen, eugenol, boric acid, attractive toxic sugar bait

I. INTRODUCTION

Female and male mosquitoes feed on plant nectars as carbohydrate sources in order to obtain the necessary energy requirements to survive. Indeed, Marshall et al. (2013) found, in laboratory studies, that some female Anopheles species will feed on sucrose daily. A number of authors have reported on the evaluation of a variety of plant sugar sources that could be incorporated into toxic baits thereby exploiting a mosquito’s sugar feeding behavior (Muller et al. 2008, Muller et al. 2010, Beier et al. 2012).

Studies of attractive toxic sugar baits (ATSB), during the past decade, have focused predominantly on integrating topically active insecticides such as pyrethroids, neonicotinoids, or organophosphates into oral formulations for adult mosquito control (Allan 2011, Muller et al. 2010, Khal-laayoune et al. 2013). In addition, boric acid and eugenol have been reported to be orally effective against several species of mosquitoes (Xue and Barnard 2003, Qualls et al. 2014, Xue et al. 2006). Interestingly, Ali et al. (2006) reported that sub-lethal doses of boric acid affected survival, blood feeding, and fecundity in Aedes albopictus (Skuse) when orally ingested. A recent laboratory study by Fulcher et al. (2014) reported effective control of adult and larval mosquitoes in runoff water from plants previously sprayed with an attractive sugar bait (ASB) and the insect growth regulator (IGR) pyriproxyfen. In addition, Chism and Apperson (2003) demonstrated 38% emergence inhibition when pyriproxyfen-treated Ae. albopictus oviposited in untreated larval habitats. Therefore, we decided to investigate the effects of sub-lethal ATSBs containing boric acid or eugenol in combination with pyriproxyfen on survival, fecundity, and fertility of the dengue and chikungunya vector, Aedes aegypti (L.).

II. MATERIALS AND METHODS

Mosquitoes. The Ae. aegypti colony used in our study, was obtained from the USDA, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. Oviposition paper containing eggs of this species was cut into 2 cm strips and placed into larval rearing pans (28 cm × 56 cm) containing 2,500 ml of reverse osmosis water (RO). This method was
utilized to produce a maximum of 500 mosquito larvae per pan. Mosquito larvae were given 3 mg of 3:2 ratio of bovine liver powder and brewer’s yeast (ICN Biomedical, Inc., OH). While in the pupal stage, mosquitoes were placed into 15 cm × 15 cm × 5 cm Ziploc brand sandwich containers (SC Johnson & Son Inc., Racine, WI) and moved into rearing cages (Bioquip Products Inc., Compton, CA) to emerge. Cotton balls saturated with 10% sucrose solution in 266 ml clear plastic cups (SOLO®, Dart Container Corporation, Mason, MI) were provided ad libitum. Adult mosquitoes were utilized in bioassays once they reached 5-7 days of age.

Attractive sugar bait (ASB). Mango fruit pulp was combined with white cane sugar at a 1:1 ratio with approximately 0.8% lime juice. The mixture was stirred and heated to 100° C and allowed to cool to 26° C, producing mango syrup. To prepare the attractive sugar bait (ASB), the syrup was mixed with reverse osmosis water at a ratio of 1:3. Green food coloring (McCormick brand) was added (0.5%) to the formulation in order to visualize ingestion by the mosquitoes (Müller et al. 2010). Also about 0.08% Agricultural Poly Control 2 Sticker Spreader (Brewer International, Vero Beach FL) was added to the ASB solution because this substance was previously used during previous field applications of ATSB (JM Scott unpubl. data). Attractive toxic sugar bait was prepared by adding toxicant to the ASB (percentages determined below).

**ATSB sub-lethal determination.** As reported by Ali et al. (2006), we selected 0.1% boric acid as the sub-lethal concentration for testing this chemical. Because biologically active sub-lethal concentrations of eugenol have not been reported, we determined this concentration is a series of ASB serial dilution bioassays. The following procedures were followed: nine to twelve female *Ae. aegypti* (5-7 days old) were mouth aspirated into individual 1 L glass mason jars, with the mouth of the jars covered with Wal-Mart brand tulle mesh secured with the metal bands of the jar lid. A total of 25 jars were used for each of 3 repetitions. Negative controls were fed ASB only. Eugenol concentrations in ASB solution were 0.1%, 0.05%, 0.025%, and 0.0125%. Green food coloring was not added to these solutions. All mosquitoes were fed about 12 ml of each solution through cotton balls placed on top of the tulle mesh. Cotton balls remained on the mesh as adult mortality was recorded at 24, 48, and 72 hours. The experiment was repeated three times. Based on the results of the eugenol bioassays, 0.0125% was selected as the sub-lethal concentration for incorporation into ATSBs (Figure 1).

**ATSB/IGR bioassays.** Boric acid (0.1%) and eugenol (0.0125%) ATSBs were separately mixed with 0.5% pyriproxyfen (P) (NyGuard® [10% AI] IGR Concentrate, Minneapolis, MN) and 0.5% P with ASB only, as the final formulations for testing. Control formulations consisted of ASB without a toxicant (negative control) and ATSB 1% boric acid (positive control). Approximately 70-80, five to seven-day old male and female mosquitoes were mouth aspirated into modified 5 L buckets with a 15 cm hole cut into the side covered by a fabric sleeve adhered with glue (Qualls et al. 2014). This sleeve allowed for continuous access to the mosquitoes, with minimal escapees. The bucket opening was covered with Wal-Mart brand tulle mesh secured with rubber bands. Each treatment was assigned randomly to each of five buckets. Baits were poured into unsalted natural collagen sausage casings, and then tied with a balloon (single) knot. Bait “sausages” were placed on top of the tulle mesh, and mosquitoes were allowed to feed for 48 hours. Adult mortality was recorded at 24 and 48 h.

**Fecundity.** Males and females (70-80 individuals, five to seven-day old) were placed together in each bucket (to ensure mating occurred) and presented with bovine blood (36° C) for 48 hours administered in the same fashion as the bait sausages. After this time, blood-fed individuals were mouth aspirated from the buckets into oviposition cages (that consisted of inverted 266 mL clear plastic cups with a 1 cm hole in the base to facilitate transfer) at the rate of one mosquito per cup. Ten cups were used per treatment including controls. This hole was stoppered with cot-
ton and utilized throughout the experiment to administer sucrose solution (10%) to the mosquitoes. The mouth of the cup was covered with tulle and secured into place with a rubber band. A 2 cm × 6 cm strip of germination paper was placed loosely into the mouth of the inverted cup by stretching the rubber band to the side and sliding the paper inside to rest on the mesh.

The oviposition cages were placed at a 45° angle into larval rearing pans (28 cm × 56 cm) that contained 1 cm of reverse osmosis water to allow the oviposition paper to be dampened. Pans were placed into an incubator and maintained at 26.6° C and 14:10 L:D. This study was repeated three times with 10 cups (females) per control/treatment and 50 cups total per repetition. Mosquitoes were allowed to oviposit for 48 hours. After this time, egg papers were collected, number of eggs counted, and allowed to dry for five days allowing for egg embryonation.

**Fertility.** Eggs were hatched in 400 ml of water in 15 cm × 15 cm × 5 cm plastic Ziploc® brand sandwich containers with lids and will be referred to as “hatch containers”. Larval food was added and lids left unscaled on one corner in order to allow for ventilation. Hatch containers were observed daily for pupae. Pupae were counted and transferred to clear plastic 266 ml plastic cups through a 0.5 cm hole in the tulle mesh covering the aperture of the cup. There were 50 cups per repetition. When not in use, the hole was covered with tape. Emerging adults were provided with a cotton ball saturated with 10% sucrose solution, ad libitum, placed on top of the mesh. Successful emergence was counted by subtracting the dead/un-emerged pupae from total pupae. Adult mortality was recorded at 48 h post initial oviposition and observed for ten days.

**Statistical analysis.** Analysis of data used JMP statistical software (SAS Institute, Inc. 2012). Mean adult survival was assessed through chi square analysis to determine significant differences between formulations. Blood feeding, and total egg data were subjected to ANOVA to determine significant differences between formulations. For all
analyses, differences were considered significant when $P<0.05$.

**III. RESULTS AND DISCUSSION**

**Adult Survival.** The ASB+P 1% boric acid formulation caused significantly greater mortality of *Ae. aegypti* at 48 h post ingestion, compared with the mortality caused by other formulations (Figure 2). Overall, none of the other ATSBs significantly affected mortality or survival of females, 10 days after oviposition, even though the 0.1% boric acid formulation resulted in >16% mortality (Figure 3). However, Ali et al. (2006) reported that a 0.1% boric acid sugar bait significantly reduced the survival rate of *Ae. albopictus* compared with an untreated control. We believe that this may be caused, in our study, by lower boric acid concentration in the ASB after mixture with pyriproxyfen.

In our initial bioassay identification of a eugenol sub-lethal concentration suitable for this study, we found that 0.1% produced significantly greater mortality compared with 0.0125% selected for our sublethal evaluations (Figure 3). These results were similar with those of Qualls et al. (2014). However, Hao et al. (2008, 2012) found that eugenol at higher concentrations significantly reduced blood feeding in adult mosquitoes and might act as a repellent. Perhaps, this may be the reason, in our study, why eugenol mixed with pyriproxyfen did not result in any significant mortality compared with controls.

**Fecundity and fertility.** No significant difference was observed between the total number of eggs laid between ATSBs and controls (Figure 4). This result was similar with the report that sublethal concentrations of boric acid sugar bait significantly reduced fecundity of *Ae. albopictus* (Ali et al. 2006), but the reduction of number of eggs in our experiment was not significant, compared with the other baits. As stated earlier, this may have been caused by the addition of pyriproxyfen.

![Figure 2. Average percent mortality of adult *Aedes aegypti* from ingestion of an attractive sugar bait (ASB) mixed with either boric acid or eugenol with 0.5% pyriproxyfen (P) after 24 and 48 h. ASB without boric acid or eugenol is an untreated control.](image-url)
to the ATSB that reduced the concentration of boric acid. However, Chism and Apperson (2003) did not observe any adverse effects on fecundity even when gravid *Ae. albopictus* were exposed to pyriproxyfen-treated surfaces.

In our study, egg hatchability considerably varied between ATSBs. In one repetition of the experiment, complete larval emergence of all eggs occurred. In another repetition, three of 25 containers with eggs did not produce larvae. In other repetitions, only a single larva was recovered from eggs produced from females that fed upon ASB+P (0.5%), ASB+P+ (0.0125%) eugenol, and ASB+P+ (0.1%) boric acid. Larvae in another container, from another repetition, died in the third instar stage (ASB+P (0.5%). We believe that the containers were possibly contaminated by an unknown contaminant, or contamination was the result of sucrose solution cotton balls where eggs had been oviposited during the evaluation.

In summary, sublethal concentrations of boric acid or eugenol in an ASB mixed with 0.5% pyriproxyfen did not significantly affect survival of *Ae. aegypti*. Also, mixing pyriproxyfen with boric acid or eugenol as baits at the concentrations used in this study did not reveal vertical transfer nor produce any significant impact on fecundity by this insect growth regulator.

**IV. ACKNOWLEDGEMENTS**

Thank you to the fellow interns and Anastasia Mosquito Control District employees who volunteered in assisting with this project and to AMCD for the opportunity to conduct the study.

**V. REFERENCES CITED**


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Figure 4. Total number of eggs laid by *Aedes aegypti* after ingestion of attractive sugar baits (ASB) containing either boric acid or eugenol with pyriproxyfen (P). ASB without boric acid or eugenol is an untreated control¹.

1(F = 0.773, df = 4,20, P > 0.05)
**ABSTRACT.** An industrial high-velocity misting fan, with and without a 1% citronella mixture, was evaluated for repelling host seeking *Aedes albopictus*. Human participants were utilized as targets and arranged in a horizontal line 5, 8, 10, and 15 meters from the fan while mosquitoes were released from the opposite end of the testing arena. Control evaluations were conducted without wind or citronella. Overall, no significant difference in participant preference by *Ae. albopictus* was observed in treatments or controls at any of the distances except at 8 m. At 15 m, significantly more mosquitoes were collected from participants with the fan on (and no mist) compared with the control (fan off). When the fan was misting 1% citronella, more mosquitoes landed on participants as distance from the fan unit increased but no statistical significant difference between the control and treatment groups occurred. In summary, the wind velocity generated by the misting fan itself, or the citronella mixture, did not significantly reduce host seeking *Aedes albopictus* and may not be a practical method of protection from biting mosquitoes for personal or public outdoor events based upon the results of our study.

**Key Words.** Misting fan, *Aedes albopictus*, citronella, host seeking

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**I. INTRODUCTION**

Mosquito-borne illnesses, such as West Nile, chikungunya, dengue, and yellow viruses, are leading global public health concerns (Kilpatrick et al. 2006, Mitchell 1991). With the advancement of widespread human transportation, these diseases and their vectors are no longer endemically confined. In addition, as human populations grow, especially in developing countries, the risk of mosquito-borne illness is becoming more threatening (Kilpatrick et al. 2006). *Aedes albopictus* (Skuse) is an important vector of several arboviral pathogens and one that is competent in transmitting many diseases in high density urban and suburban settings because of widely available larval developmental areas in close proximity to human habitation (Mitchell 1991).

A recent report claiming that an oscillating table fan was capable of deterring mosquito bites at a backyard gathering by disrupting a mosquito’s ability to locate potential hosts has provided additional interest in non-chemical and natural repellent options (Broad 2013). Fans have commonly been used to deter pest insects, such as cockroaches and fruit flies in homes and businesses (Kolbe 2003). The American Mosquito Control Association describes most mosquitoes as weak fliers, only capable of flying at 0.45 to 0.67 meters per second, and recommends placing a large fan on a household deck or patio to provide a “low tech solution” to reduce mosquito bites (AMCA, 2014). Manipulating wind as a means of repellency is gaining more public attention and remains relatively unstudied.
Citronella oil is a widely recognized and commonly utilized insect repellent. Recent studies have debated the efficacy of citronella to repel mosquitoes (Tawatsin et al. 2001, Yang and Ma 2005, Hao et al. 2008, Gross and Coats 2015). Multiple studies have hailed the success of combining wind currents with vapor or mists of the repellent, DEET (Hoffman and Miller 2002, Hoffman and Miller 2003), the spatial repellent, metofluthrin in a clip-on fan device (Xue et al 2012, Revay et al 2013), or geraniol and citric acid in ALLCLEAR Backyard Mosquito Misters (Revay et al 2013a) for use in reducing mosquito bites. However, there is lack of evidence that commercial mechanical fans misting citronella can repel the container-inhabiting mosquito, *Ae. albopictus*. Our study objectives were to 1) determine the effectiveness of a high powered commercial misting fan to deter *Ae. albopictus* from host-seeking with wind currents at multiple distances from the machine, and 2) test the effectiveness of the fan to prevent mosquito bites when misting a 1% citronella oil at multiple distances from the machine.

II. MATERIALS AND METHODS

Power Breezer® machines (Breezer Holdings LLC, Deerfield Beach, Florida) were acquired from the manufacturer for testing. These industrial fans were equipped with a 380 L (100 gal) tank and locking rear casters to secure the fan in place. Another feature that was utilized in this study was the atomizer capable of producing a fine mist from the solution in the tank. The unit was evaluated at Anastasia Mosquito Control District (AMCD) district headquarters in a 12.2 × 18.3 m metal garage previously cleaned and sealed for use as the experimental arena. The Power Breezer was situated in the northwest corner of the garage and oriented with the fan-facing the opposing southeastern corner. A 24 m line was measured from the

![Figure 1. Orientation and average wind velocity at multiple distances of the Power Breezer machine during experimental trials.](image-url)
fan to the opposite corner of the building with lines marked with blue painters tape at 5 m, 8 m, 10 m, and 15 m (Figure 1). For each distance, six points were marked 0.6 m from one another that represented where each participant would stand for evaluation.

Average wind speed of the Power Breezer was determined at its highest setting using a handheld SKYWATCH® Xplorer 3 anemometer (JDC Electronics SA, Yverdon-les-Bains, Switzerland). Speeds were recorded at angles left, right, and center of the machine at the 5 m, 10 m, and 15 m distances. At 8 m, only wind speed at the center point was measured. Average wind-speeds are listed in Figure 1. The garage bay door closest to the unit remained open to alleviate potential pressure buildup in the building generated by the high-powered fan. The misting unit flow rate averaged 100 ml/min for each ten-minute trial.

Two hundred, caged, adult 5-7 day old laboratory reared female *Ae. albopictus* were brought to the testing area and placed at the opposite corner, 24 m from the fan unit. For each test, mosquitoes were allowed to acclimate to the new environment for up to 30 minutes. Six participants (3 males, 3 females) were used in the study and did not rotate during evaluations. Testing began at the farthest distance from the Power Breezer (15 m). Mosquitoes were released and given ten minutes to actively host seek participants. Mosquitoes that came to the participants, during that time were killed and counted. At the end of the evaluation period total mosquito landings were recorded. New mosquitoes were used for each distance tested. Each objective was evaluated only one time.

Objective one, evaluated the fan on without mist, while in the second objective the fan applied a 1% citronella oil in water mist. Testing distances were reduced to the 10 m and 15 m points in the citronella trials due to insufficient landing numbers in previous trials at the 5 m and 8 m distances. Control trials were conducted with the fan off and without citronella. Objective one was conducted in April of 2014, while objective two was conducted in June 2014. During each objective, new participants were used for controls and treatments based on availability.

**Statistical analysis** All data were analyzed by ANOVA using JMP® software (SAS Institute Inc. 2013). Overall, host-seeking abundance of *Ae. albopictus* between participants was compared within pooled controls and pooled treatment groups to determine if differences occurred. The average number of mosquitoes able to locate participants in control (no wind) and treatment wind trials (with and without citronella) at each distance from the Power Breezer were compared. Significant differences were determined at *p* < 0.05.

### III. RESULTS

No significant difference in the number of *Ae. albopictus* recovered between participants at each sample distance occurred with the exception of 8 m where significantly more mosquitoes were collected (15 m: *F* =0.1757, *p*=0.9620; 10 m: *F*=1.2236, *p*=0.4005; 8 m: *F*=4.6032, *p*=0.0451; 5 m: *F*=1.9314, *p*=0.2223) (Figure 2). During the first objective, there were no observed difference between the treatments and controls at 5, 8, 10 meters from the Power Breezer fan (5 m: *F*=2.2321, *p*=0.1660, 8 m: *F*=0.0440, *p*=0.8381, 10 m: *F*=2.4806, *p*=0.1463). At 15 m significantly more mosquitoes were collected from the treatment than the control (*F*=23.416, *p*=0.0007) (Figure 2).

When the fan was misting 1% citronella, statistically more mosquitoes landed on participants as distance from the fan unit increased (*F*=18.2826, *p*=0.0003) (Figure 3). There were no observed difference between treatments and control at either distance from the Power Breezer fan (10 m: *F*=2.5966, *p*=0.1382 and 15 m: *F*=0.2062, *p*=0.6595).

### IV. DISCUSSION

The significant differences that we found between the participants at 8 m in
Thomson et al.: Evaluation of Power Breezer and Misting Citronella Against *Aedes albopictus*

控制和治疗组，并在15米的距离上，风的风力强度可能对无香茅试验组的蚊子有影响。这一影响可能归因于测试区域内的各种随机变量。每次测试之间的时间间隔以及在部分封闭空间中的温度可能会对试验结果产生影响。此外，试验参与者也被允许主动弯曲，蹲下，以及物理上呼出二氧化碳以吸引蚊子。在15米处，参与者距离释放点只有9米远，而距离风扇最远的最弱风在此处发生。低风
velocity from the fan may have facilitated host-seeking behavior by enhancing dispersal of a participant’s kariomones making the mosquitoes to be more attractive to their host.

Hoffman and Miller (2003) analyzed the relationship of wind velocity with mosquito host seeking and flight behavior. The authors reported that wind likely had the greatest effect on host odor dilution for host seeking mosquitoes. This mechanism outweighed the relationship of higher wind velocity, that can create intolerable flying conditions for the mosquito. The high velocity wind stream produced by the Power Breezer exceeded the 0.45 m/s flying capability of mosquitoes at every test distance. But we found that wind alone did not significantly deter host seeking mosquitoes as pointed out by Snow (1977) and Hoffman and Miller (2002). We found that the wind stream from the Power Breezer appeared to produce a boundary layer of calmer conditions lower to the ground and may have facilitated favorable conditions for mosquito up wind flight to participants after host location.

In an earlier study, Hoffman and Miller (2002) focused on manipulating wind currents while combining a DEET mist and found this treatment provided temporary protection from mosquitoes. These results are in contrast with our study with citronella. The differences in both studies may be attributable to the chemicals used and possibly concentrations. Most laboratory studies that test repellency of citronella use concentrations ranging from 10-30%, and often include 5% vanillin to enhance repellency (Tawatsin et al. 2001, Yang and Ma 2005, Hao et al. 2008) while we only applied 1% citronella. In summary, wind velocity generated by the Power Breezer fan itself, or with the citronella mixture, did not significantly reduce host seeking *Ae. albopictus* and may not be a practical method for preventing mosquito bites for personal during public outdoor events.

V. ACKNOWLEDGEMENTS

This is a research report only and does not imply AMCD endorsement of any commercial products. Volunteers gave informed consent prior to participation in the study. This study was conducted according to protocol number AMCD-10-13-2005 as approved by the AMCD Board of Commissioners for use of human subjects in the operation of projects.

VI. REFERENCES CITED


ABSTRACT. Talstar® Professional (7.9% bifenthrin, AI) was applied by a Stihl SR 420 gas powered backpack sprayer to the perimeter vegetation of an 11 ha cemetery in order to control the source of adult *Aedes albopictus* into the surrounding residential neighborhoods. Prior to, and after treatment, BG Sentinel traps baited with BG-Lure were used to monitor adult abundance in the cemetery while egg production was monitored using black oviposition cups. Surveillance continued for six weeks post treatment with no additional treatments applied to the site. Adult Asian tiger populations were significantly reduced in the cemetery one week after treatment (97.75%) compared with areas with no control measures. Population control declined, thereafter, to about 43% at four weeks post treatment and was still significantly reduced despite a total rainfall of 12.7 cm (with a peak of 6.4 cm) during the study period. The trend in egg reduction from oviposition traps mirrored that of the adult mosquito population. We found that the barrier treatment was an effective method for controlling *Ae. albopictus* where other methods, especially source reduction, have limited utility.

Key Words. *Aedes albopictus*, barrier spray, bifenthrin, cemetery, residential area

I. INTRODUCTION

The Asian tiger mosquito, *Aedes albopictus* (Skuse), is considered one of the most abundant and invasive anthropophilic mosquitoes commonly encountered in urban environments (Lounibos et al. 2001). The ubiquity of this species, especially when in association with human environs, poses significant problems as the result of its successful ability to colonize cryptic oviposition sites and rapidly produce large populations. Moreover, *Ae. albopictus* is a known vector of multiple arboviruses, including chikungunya (CHIKV), dengue, and the recent Zika virus (Derraik and Slaney 2015, Ngoagouni et al. 2015, Wilson and Chen 2015). In 2014, the Florida Department of Health confirmed locally acquired cases of CHIKV from several counties.

Cemeteries provide a good example of cryptic environments suitable for *Ae. albopictus* production and harborage. These areas are often large sites with static waterholding containers such as flower urns built into, or mounted on, head stones. This environment often contains extensive patches of high density vegetation such as bamboo thickets that can create small containers for mosquito larvae when pruned back (Washburn and Hartmann 1992, Johnson and Sukhdeo 2013). This situation can lead to highly localized, but heavy, outbreaks as adult *Ae. albopictus* host seek during the day. Source reduction is often a principle method in eliminating oviposition and larval production habitat for this species but may be ineffective in some instances.

Therefore, the best approach for building a repertoire of effective management strategies to reduce *Ae. albopictus* populations and/or the transmission risk of arbo-
viral infection is through integrated vector management (IVM). One method, with decades of usage, is barrier spraying (Trout et al. 2007, Qualls et al. 2013). The Anastasia Mosquito Control District (AMCD) is familiar with this method and has used it to successfully control adult mosquitoes (Qualls 2013). Other techniques, such as thermal fogging with adulticides, have been reintroduced into AMCD operational programs for control of adult container-inhabiting mosquitoes in order to bolster the options for IVM (Xue et al. 2013). But just having the technologies available for use is not enough. Chemical application methods, either singular or in combination, must be continually re-evaluated for their applicability to contemporary problems of vector abatement in order to stay one step ahead of emergent disease threats like CHIKV, dengue, and Zika viruses. However, any tactics for long-term control of Ae. albopictus populations would be welcomed.

As mentioned earlier, pesticides applied to vegetation as a residual barrier for adult mosquito control can provide several versatile approaches for mosquito management. A study by Cilek (2008) showed that bifenthrin applied to the perimeter vegetation of a public park provided effective (>70%) control against adult Ae. albopictus and Culex quinquefasciatus Say, for six weeks, in residual leaf bioassays. Using this approach we tested the efficacy of insecticide barrier treatments to reduce Ae. albopictus populations in a cemetery surrounded by an urban residential network of neighborhoods. These neighborhoods shared a fence line perimeter with the cemetery and have experienced considerable annoyance from host seeking Ae. albopictus originating from the latter area.

II. MATERIALS AND METHODS

**Test Site:** A local 11 ha cemetery (latitude 29.893712, longitude -81.337268) in St. Johns County was targeted as a major source of Ae. albopictus production. This area was surrounded on all sides by urban residential housing with high human activity. The property was chosen for its abundant larval sites for which source reduction education has had little impact. Larval developmental habitats in the cemetery included dense bamboo thickets, flower urns, exposed crypts, and ground maintenance storage areas overloaded with tire piles. These habitats were routinely irrigated as part of normal ground maintenance. Mosquito landing rate counts were conducted in the cemetery for three minutes in 18 locations to identify where the majority of Ae. albopictus activity occurred. From this information, four locations were chosen for the study and monitored from June 1 through July 25, 2015.

**Surveillance.** Two BG Sentinel™ traps (BGS) baited with a standard Biogents® BG-Lure (Biogents AG, Regensburg, Germany) were placed at least 50 m apart in each location (Figure 1). Traps were operated continuously for 48 h per week for two weeks prior to treatment. In addition, five 500 ml black oviposition cups, lined with velour oviposition paper, were placed in the same four locations and allowed to collect eggs for 48 h per week during the same period. After treatment, both surveillance methods were continued in the same frequency for six weeks. Rainfall was recorded weekly. Collections from BGS traps and ovicup papers were transported to the lab. Captured mosquitoes were identified to species and eggs from ovipapers were counted using a light dissecting scope.

**Barrier treatment.** The outer perimeter vegetation and subsequent fence line of the entire 11 ha site was treated with Talstar® Professional (7.9% bifenthrin AI, Philadelphia, PA) at a dilution rate of 11.4-L water to 88.7 ml bifenthrin. Treatment was applied using a Stihl SR 420 gas powered backpack sprayer (Andreas Stihl Ag & Co. KG, Waiblingen, Germany) at a 3.3 L flow rate while walking the area at 6.4 kph. Application height was at 3 m with a 60 m penetration depth for a total treatment area of 6400 m². The pesticide application was carried out by a certified State of Florida Public Health Pest Control applicator.
Statistical analysis. Percent population reduction was calculated using a proportion derived by $T/(T+P)$ where $T =$ the population for a given week of treatment and $P =$ the population for the two weeks of pre-treatment sampling. Statistical software JMP® version 10.1 (SAS Institute, Inc. 2012) was used to run a paired $t$-test on mean adult and egg surveillance data to determine differences in population level from the treatment compared with pretreatment level per week for 6 weeks.

Ethics statement. Those volunteers who participated in landing rate counts were briefed on the study procedures and made aware of the health risks involved by their participation. Written informed consent was received from volunteers prior to the start of the study. Volunteer consent is documented under the AMCD IRB Protocol #10.13.2005 and approved for use of human subjects by the Board of Commissioners presiding over the AMCD.

III. RESULTS AND DISCUSSION

*Aedes albopictus* populations were significantly reduced by 97.75% during week one; 65.64% during week two; 58.72% for week 3; and 43.39% at week 4 (Figure 2). Population levels at weeks 5 and 6, post treatment, were no longer significantly different than pretreatment levels. Egg abundance after treatment was also significantly reduced ($t = 4.7154$, df = 3, $p < 0.0181$) for the first four weeks.

Despite such a large source site, at 11 ha, the perimeter barrier spray still provided successful control of the resident *Ae. albopictus* population without additional adulticide or larvicidal treatment. Previously, this area served as an important source of adult Asian tiger mosquito for neighborhoods surrounding it. The cemetery is a largely unmanaged property compounded by dense bamboo privacy screens along the perimeter. Addi-
Aedes albopictus pre- & post-treatment surveillance

![Graph showing weekly mean precipitation and abundance of adult Aedes albopictus from BG Sentinel (BG AVG) and mosquito egg oviposition cups (EGG AVG) in an 11 ha cemetery before (week 1-2) and after (week 3-8) treatment of perimeter vegetation with Talstar Professional (bifenthrin). Vertical lines at each data point are standard errors.]

Figure 2. Weekly mean precipitation and abundance of adult Aedes albopictus from BG Sentinel (BG AVG) and mosquito egg oviposition cups (EGG AVG) in an 11 ha cemetery before (week 1-2) and after (week 3-8) treatment of perimeter vegetation with Talstar Professional (bifenthrin). Vertical lines at each data point are standard errors.

tionally, tire piles and human generated debris that included artificial containers expanded the available sources. As stated earlier, traditional, methods of source reduction and custodial education of cemetery employees on identifying and removing urban mosquito sources were met with resistance. Moreover, relatively few roads for truck access into the cemetery limited the effectiveness, and increased the cost of any larviciding or adulticiding effort often requiring multiple visits over a season. Barrier spraying with Talstar Professional has shown, in this case, to be the most effective means of reducing large urban sources of adult Aedes albopictus.

IV. ACKNOWLEDGEMENTS

Appreciation is extended to the custodians of the cemetery site who collaborated with the test evaluation. The operational technicians of the Anastasia Mosquito Control District are thanked for their collaboration during this large barrier mission. This is a research report only. The AMCD does not endorse any commercial mentioned in this report.

VI. REFERENCES CITED


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LABORATORY EVALUATIONS OF SEVEN INSECT REPELLENTS AGAINST THE LONE STAR TICK

AMBLYOMMA AMERICANUM

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ABSTRACT. In laboratory bioassays, BioUD, Bio Block-Organic Outdoor, Bio Block-Organic Pest Control, Bio Blocker-Organic Insect Repellent, OFF! Botanicals Insect Repellent, OFF! Deep Woods Insect Repellent, and Repel Insect Repellent Sportsman Gear Smartinsect repellents were applied to paper and assessed by four volunteers for efficacy against unfed nymphal Amblyomma americanum ticks. Assessment times of 10 and 120 minutes post application were chosen to mimic freshly applied repellent and suggested duration of effectiveness against ticks as determined by the product manufacturers. Significant differences between repellents existed when both application periods were compared but not between volunteers. At 10 min post application, Bio Block Pest Repel was the most effective product at repelling ticks (85%) and Repel the least effective (30%). At 120 min, BiteBlocker-Insect Repel, OFF! Deepwoods, and Bio Block-Organic Outdoor provided ≥55% protection from ticks. Efficacy at 120 min, when measured at 50% or greater repellency, did not reflect duration times listed on the BioUD (20%), and OFF! Botanicals (30%) product labels.

Key Words: Insect repellents, DEET, ticks, Amblyomma americanum

I. INTRODUCTION

Amblyomma americanum (L.), the lone star tick, is a commonly encountered tick in northeastern Florida. Annually, Anastasia Mosquito Control District (AMCD) receives multiple customer complaints about this tick species and is often asked for recommendations on how to avoid tick bites. In addition, AMCD’s field technicians frequently encounter these ticks when conducting fieldwork. Topical and barrier insect repellents offer an inexpensive mode of protection that imposes the Fewest limitations on human activities (Staub et al. 2002). With the possible transmission of tick-borne pathogens, such as Ehrlichia chaffeensis, E. ewingii, E. muris, Southern Tick-Associated Rash Illness (STARI), and Francisella tularensis (Mixson et al. 2006, CDC 2015), as well as the growing concerns over DEET (N,N-diethyl-3-methylbenzamide) based products on the market (such as, smell, possible bioaccumulation, and damage to plastics and some clothing materials) as reported by Katz et al. (2008) and Semmler et al. (2011), alternative tick repellents should be evaluated. Therefore, a laboratory study was conducted to determine the repellent effectiveness of several commercially available non-DEET products against lone star ticks.

II. MATERIALS AND METHODS

Insect repellents. Seven DEET and non-DEET commercially available products were chosen to provide diversity of active ingredients that may be repellent to A. americanum (Table 1). HOMS products were supplied to AMCD for evaluation of efficacy by the manufacturer, while other repellents were purchased at local pharmacies or hardware stores. HOMS Bio Block Organic Outdoor and HOMS Bio-Block Organic Pest Control are designed for premise treatment while the rest of the products that we tested were labeled for application to human skin.
Human volunteers. Male (2) and female (2) volunteers were utilized in these evaluations. Ages of the volunteers ranged from 24-55 years old and signed consent forms under AMCD’s IRB protocol#10-13-2005 (as approved by the AMCD Board of Commissioners for studies involving use of human subjects).

Ticks. Non-blood fed, nymphal *A. americana* were obtained from a laboratory colony maintained by USDA/ARS, Tick and Biting Fly Research Unit, Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, TX. New ticks were used by each volunteer during each repellent evaluation. After individual volunteers evaluated repellency, the ticks were collected by scotch tape and disposed of.

Bioassays: The bioassay utilized a modification of human skin test evaluations used by Semmler et al. (2011). White poster paper (1 meter x 1 meter) was used as a substratum for all repellent applications. Poster boards were taped onto individual tables (183 cm x 76 cm x 74 cm) located one meter from each other. Different concentric circles were drawn onto the poster boards: 15.5 cm outer circle, 14.5 cm middle circle, and 5.5 cm inner circle. Following label instructions for application, repellents were painted between the 15.5 cm and 14.5 cm circles and allowed to dry for ten minutes. After ten minutes, 5 ticks were placed with forceps onto the center of the 5.5 cm circle. Volunteers sat in a chair at each table and placed their arms to the left and right of the outer 15.5 cm circle, ensuring no contact with repellent. Ticks were given 3 min to locate volunteers. Successful location was recorded when ticks crossed the 5.5 cm center circle and came into contact with the 14.5 cm middle circle. If ticks did not locate volunteers within 3 minutes, they were collected with scotch tape, and the next volunteer evaluated the same repellent using new ticks. Each repellent was evaluated in this manner. A secondary evaluation, 120 minutes after initial application, was conducted with the same repellent as in the initial evaluation. A total of 60 repellent evaluations were conducted.

Table 1. Manufacturer, active ingredient (%), commercial product, formulation and manufacturer’s stated duration of protection.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Active Ingredients1</th>
<th>Brand Name</th>
<th>Duration of protection</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMS, LLC</td>
<td>2-undecanone 7.75%</td>
<td>BioUD®</td>
<td>120 minutes</td>
<td>Pump spray</td>
</tr>
<tr>
<td>HOMS, LLC</td>
<td>Soybean Oil 20%, Citric Acid 4%, Cedar Oil 1%</td>
<td>Bio Block®-Organic Outdoor</td>
<td>Outdoor-Monthly</td>
<td>Concentrate</td>
</tr>
<tr>
<td>HOMS, LLC</td>
<td>Soybean oil 12%, Geranium oil 3%, Cedar oil 1%, Lemon Grass oil 0.5%</td>
<td>Bio Block®-Organic, Pest Control</td>
<td>Indoor-Monthly, Outdoor-Weekly</td>
<td>Pump Spray</td>
</tr>
<tr>
<td>HOMS, LLC</td>
<td>Soybean Oil 30%, Citric Acid 4%, Cedar Oil 1%</td>
<td>BiteBlocker®-Organic, Insect Repellent</td>
<td>120 minutes</td>
<td>Pump spray, Lotion, Lotion, Travel pen</td>
</tr>
<tr>
<td>SC Johnson &amp; Sons</td>
<td>p-Menthane-3,8-Diol 10%</td>
<td>OFF!-Botanicals® Insect Repellent</td>
<td>120 minutes</td>
<td>Pump spray</td>
</tr>
<tr>
<td>SC Johnson &amp; Sons</td>
<td>DEET 25%</td>
<td>OFF!-Deep Woods, Insect Repellent</td>
<td>Not listed</td>
<td>Aerosol Spray</td>
</tr>
<tr>
<td>WPC Brands, Inc.</td>
<td>Picardin 15%</td>
<td>Repel®-Insect Repellent, Sportsman Gear Smart</td>
<td>480 minutes</td>
<td>No longer available</td>
</tr>
</tbody>
</table>

1 Brand name formulations are subject to change; labels should always be read to ensure exact ingredients.
2 N,N-diethyl-3-methylbenzamide

**Notes:**
- Ages of the volunteers ranged from 24-55 years old and signed consent forms under AMCD’s IRB protocol#10-13-2005 (as approved by the AMCD Board of Commissioners for studies involving use of human subjects).
- Ticks. Non-blood fed, nymphal *A. americana* were obtained from a laboratory colony maintained by USDA/ARS, Tick and Biting Fly Research Unit, Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, TX. New ticks were used by each volunteer during each repellent evaluation. After individual volunteers evaluated repellency, the ticks were collected by scotch tape and disposed of.
- A total of 60 repellent evaluations were conducted.
followed the previously outlined bioassay. Ticks on the control poster board followed the same bioassay procedure as the treated ones.

**Statistical analysis.** The statistical software JMP version 11.1.1 (SAS Institute Inc. 2013) was used for data analysis. Statistical significance was determined through ANOVA ($P<0.05$). Post hoc analysis was conducted to separate the means of repellents using Tukey HSD.

### III. RESULTS

Our study focused on insect repellents that offered a wide variety of active ingredients and evaluated their efficacy when mimicking freshly applied at 10 min and at 120 min post application to mimic the label duration of effectiveness listed by most of the manufacturers. Significant differences existed between products but not between volunteers, at both post application time periods ($F = 5.1137$, $df_{10,21} = 31$, $P = 0.0008$, and $F = 4.0310$, $df_{10,21} = 31$, $P = 0.0034$, respectively) (Table 2). At 10 min post application, Bio Block Pest Repel was the most effective product at repelling ticks (85%) while Repel was the least effective (30%). At 120 min, Bite Blocker Insect Repel, OFF! Deep woods, and Bio Block-Organic Outdoor provided ≥55% protection from ticks and was consistent with their respective labeled protection rates as listed in the EPA Insect Repellents Use and Effectiveness guidelines (EPA 2015). At 120 min, the efficacy of BioUD (20%), and OFF! Botanicals (30%), measured at 50% or greater repellency, did not reflect labeled duration times listed on their labels. We believe these studies have provided several options, for the general public and our field technicians, on what commercially repellents are efficacious and available for protection against nymphal host seeking *A. americanum*.

### IV. ACKNOWLEDGEMENTS

All volunteers gave informed consent regarding the possible risks of acquiring tick-borne diseases during the laboratory trials. This is a research report only, use or mention of trade names does not constitute an official endorsement by Anastasia Mosquito Control District.

### V. REFERENCES CITED


LABORATORY AND FIELD EVALUATION OF OFF! CLIP-ON MOSQUITO REPELLENT DEVICE CONTAINING METOFLUTHRIN AGAINST THE LONE STAR TICK, AMBLYOMMA AMERICANUM (ACARI: IXODIDAE)

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ABSTRACT. Choice tests were conducted in the laboratory to determine the repellency of the pyrethroid insecticide metofluthrin (Off!® Clip-on™ Mosquito Repellent, AI 31.2%) to lone star ticks, Amblyomma americanum. In this study, the device’s fan unit was disabled to allow evaluation of the active ingredient as a passive spatial repellent. We found that 57% of the ticks were significantly repelled from hands that held the Off! Clip-on refill, compared with 27% of volunteer hands without it (control). Field trials conducted in northeastern Florida with volunteers either sitting or walking while wearing the repellent device in areas infested with A. americanum were also evaluated. Seated volunteers, with the device attached to their waist, with the fan running repelled 89% of host-seeking ticks when compared with persons similarly placed without the fan on. In a second field trial, volunteers that walked slowly through the naturally infested area with the repellent fan running provided 28% protection from questing ticks compared with similar volunteers without the fan on. Our overall results demonstrated that fewer numbers of lone star ticks were found on persons with the Off! Clip-on Mosquito Repellent device operating compared with those wearing the device in the off position, regardless of sitting or walking slowly through a tick infested area.

Key Words. Amblyomma americanum, tick repellent, metofluthrin, personal protection, Off Clip-on Mosquito Repellent

I. INTRODUCTION

Tick-borne diseases in humans and animals are a growing world-wide concern and problem (Gratz 1999). A common tick in the southeastern US is the lone star tick, Amblyomma americanum (L.) and has been reported to be a potential vector of human monocytic ehrlichiosis as well as several other emerging tick-borne pathogens (Childs and Paddock 2003). Anastasia Mosquito Control District clientele, and our field staff, have experienced numerous encounters with this pest tick species after visiting some of the state parks in St. Johns County, Florida (Xue, pers. comm.). Tick control using an acaricide is very difficult in Florida state parks because these areas are considered conservation lands where pesticide applications are prohibited.
Therefore, personal protective measures rely on topical application of repellents as the most effective protection against host-seeking ticks (Carroll et al. 2010).

Most common commercially available repellent products contain different concentrations of N, N-diethyl-3-methylbenzamide (DEET) as the active ingredient (Xue et al. 2007). Due to concerns about DEET’s potential toxicity and safety (Osimitz and Grothaus 1995), including possible health risks associated with repeated use of high concentrations (Moody 1989), spatial repellents have been suggested as alternatives. Theoretically, an effective spatial repellent should provide an envelope of protection that prevents contact between host and pest while preventing pathogen transmission if disease vectors are present (Achee et al. 2012). Most arthropod spatial repellent products release an active ingredient as a vapor or smoke. Both dispersal methods often use a heat source, or fan, to produce the protection zone. Metofluthrin, a vapor-active pyrethroid, has been identified as an effective spatial mosquito repellent (Ujihara et al. 2004, Kawada et al. 2005). Several novel methods of applying spatial repellents against mosquitoes have included impregnated paper (Argueta et al. 2004; Lucas et al. 2007) and plastic strips (Kawada et al. 2008). Recently, Off!® Clip-on™ Mosquito Repellent was introduced as a new commercially-available spatial repellent device; this product has been tested against mosquitoes and provided effective protection in northeastern Florida (Xue et al. 2012). To date, Off! Clip-on has not been evaluated (nor its active ingredient) for personal protection against ticks. Here we report the results of laboratory and field evaluations conducted in northeastern Florida, to determine the repellency of Off! Clip-on Mosquito Repellent against host-seeking adult and nymphal *A. americanum*.

II. MATERIALS AND METHODS

*Spatial repellent device.* Off! Clip-on Mosquito Repellent devices were purchased from a local convenience store. This device consists of a plastic dispenser containing a replaceable cartridge with metofluthrin (AI 31.2%). This product is designed to operate by turning on a fan powered by two 1.5 volt AA alkaline batteries to assist in the release of the active ingredient into the air surrounding the individual. According to the label, the device can be worn by adults or children for protection against mosquitoes for up to 12 h of operation.

**Laboratory test.** Static, non-fan-mediated activity of metofluthrin was evaluated in laboratory cage bioassays. Each cage was constructed from a plastic Sterilite® box (13 × 13 × 21.5 cm; L × W × H, Sterilite, Townsend, MA) and served as an open airflow chamber (OAC) for choice evaluations (Figure 1). A clear vinyl tube (3.5 cm outer diameter × 30 cm) was connected to the OAC through a 3.5 cm diameter hole on the side. The tube was then connected to a 1,000 ml polystyrene disposable storage bottle (Corning®, Corning, NY) through a 3.5 cm diameter hole in the center of the bottle lid. The base of the bottle had been removed to allow insertion of a volunteer’s hand.

Laboratory-reared (non-blood fed) adult *A. americanum* were used in the study from a colony maintained by the USDA/ARS, Tick and Biting Fly Research Unit, Knipling-Bushland U.S. Livestock Insect Research Laboratory, Kerrville, TX. For each test, five ticks were placed into the vinyl tube, using feather tip forceps, and secured with fabric mesh held in place by rubber bands to ensure that no ticks escaped the tube. Ticks were allowed 2 min to acclimate in the tube before it was attached to the OAC and polystyrene bottle. A volunteer then placed one hand into the end of the polystyrene bottle and ticks were given 5 min to move to their final position. After five minutes, ticks located between the hand, on the mesh, and up to 15 cm from the hand were counted as “host seeking”. Ticks were given a 2-min rest period then the assay repeated but with the volunteer holding an OFF! Clip-
on refill while placing their hand in the polystyrene chamber. Ticks were allowed 10 min to locate the volunteer’s hand. After 10 min, ticks located on the mesh next to the hand, and within 15 cm of the hand, were counted as attracted to the host. For all tests, the percentage responding was calculated. Three volunteers conducted three sessions each providing three replications with an N = 9. New ticks were used for each replication.

Field test. Field trials were conducted at Guana State Park (permit# 2012-3-12) in South Ponte Vedra, Florida (30° 07” 56.37”N and 81° 22” 40.44 W). This land parcel is a conservation area where large populations of lone star ticks occur. Six human volunteers (four males and two females) who each signed a consent form under protocol No. 445-96, as approved by the University of Florida, Institutional Review Board (IRB-01) participated in the trials. Volunteers wore white clothing that fully covered their body from head to toe. Each person had one Off! Clip-on device attached to their waist and sat on a chair 15 m away from the nearest volunteer.

Three volunteers served as treatments, with their device fan turned on for 30 min. During the same time period, the three remaining volunteers had an Off! Clip-on device attached to their waist but without the fan operating (referred to as “static device”) and served as controls. All stages of ticks that climbed upon the volunteers clothing were collected by scotch tape by the end of the observation period. All volunteers then relocated to a new testing site in the same area and switched treatments, (i.e. previous control volunteers switched to serve as the treatment and the previous treated volunteers now served as the control). This scenario was repeated 6 times on one day during June 2012. Meteorological data (wind direction, speed, air temperature, and relative humidity) were collected hourly with a small hand-held digital meter (Skywatch Xplorer, N Tech, USA, Holmen, WI).

In the second field trial at the same state park, six volunteers (3 females and 3 males) participated. Three volunteers wore devices, attached to their waist, without operating the fan while the remaining
three volunteers wore devices where the fan was operated. Volunteers wore the previously mentioned white suits from the first trial and walked at a pace covering 30 m for 15 min. Each volunteer was positioned 15 m from the next volunteer. Ticks were removed from volunteers by the end of each time period. After the 15 min collection period, volunteers relocated to a new test site and either switched on or off their fans, to provide a comparison to their previous treatment assignment. These field trials were repeated six times on one day from 0800 to 1500 during late May, 2012.

Data analyses. Mean percent response data from the laboratory study were subjected to an analysis of variance using JMP version 11 (SAS Institute, Inc. 2013) on non-transformed data, to assess statistical significance between controls and treatments on pooled data across all volunteers. Tukeys HSD was then conducted on this dataset to determine if differences existed (P<0.05). Field trial repellency data was evaluated in a split-plot design. The data for walking and sitting were analyzed separately. Mean tick counts were transformed using square root \((x + 1)\) prior to analyses and Student’s t-test performed to determine differences (P<0.05).

III. RESULTS

Laboratory test. There was statistical significance between the treatment and the control \((F_{1,36} = 9.7826, P = 0.0065)\). Fifty-seven percent of ticks were repelled from the volunteer holding the clip on refill, while 27% ticks were repelled from the volunteer without holding the clip on refill. There was no statistical difference between volunteers in these trials \((F_{2,15} = 0.3439, p = 0.7144)\).

Field test. Only one tick species, A. americanum, was collected in the field trials. A total of 98 ticks were collected including 6 females, 3 males, and 89 nymphs. At 30 min, a mean of 3 ticks were recovered from seated volunteers holding the clip on devices. During the same time period, 2 ticks were recovered from only one of the six volunteers with operating devices for a mean of 0.33 ticks. In addition, 2-6 ticks were noticed around the feet of the three sitting volunteers while the fan was on but they did not climb onto either person. During this part of the field study, 89% protection was provided by the clip-on device operating against questing ticks for volunteers who were sitting. Wind speed during the trial was recorded at 0-9 km/h with air temperature ranging between 25 and 28° C (70% RH).

At the beginning of the walking field trial, numerous ticks were initially observed on the feet and legs of all volunteers. However, those with the repellent device turned on noticed that those ticks either left or dropped off each person while the device was operated for a few minutes. A mean of 7.3 ticks per person per 15 min were observed on volunteers with the device off and 5 ticks per person per 15 min on persons with an operating device. The percent protection from questing A. americanum afforded to walking individuals by operating OFF! Clip-on device was 28% compared with persons with a non-operational device. In this field trial, there were no significant differences in the numbers of ticks recovered between volunteers when using, or not using, the fan component. During this study wind speed was recorded at 0-3.6 km/h with air temperature ranging from 26–28° C (70% RH) in the morning of the second field trial.

IV. DISCUSSION

Host-seeking ticks can approach a host by walking or they may sit and wait for the host to make contact, a process called questing. Usually questing is the primary host seeking behavior for ticks rather than directly approaching a host. Therefore, walking is an ideal method to evaluate tick repellents for those species that primarily utilize questing to acquire hosts. Because few ticks actively move toward a host, and most ticks walk/approach a host very slowly, sitting for 15 min periods may be a less
found that operating an Off! Clip-on mosquito repellent device containing metofluthrin proved to be more effective (89%) for protecting inactive (sitting) hosts from climbing ticks than it was for hosts that were actively walking (28%). This effect was most likely influenced by entering tick harborage areas and the tick’s ability for host location through its questing behavior. To provide more effective protection for individuals walking in tick infested habitats, the Off! Clip-on device needs to be improved by possibly increasing the concentration of metofluthrin, fan speed, and/or wearing the device on the lower part of legs rather than on the waist.

Although the Off! Clip-On Mosquito Repellent was designed to primarily repel mosquitoes, we observed that a certain amount of effectiveness against crawling A. americanum on clothing can be achieved. Moreover, we found that the Off! device provided effective protection from crawling ticks especially when people sit outdoors.

The Off! Clip-on Mosquito Repellent system is easy to operate and can be attached on a belt, purse, or placed on a flat surface for protection against host-seeking arthropods (Xue et al. 2012). The active ingredient, metofluthrin, can last for up to 12 h (for mosquitoes) and used multiple times but must be refilled within 14 days after opening according to the manufacturer. However, the actual longevity of the active ingredient in this device (measured in hours or days) as personal protection against host-seeking ticks has not been evaluated.

V. ACKNOWLEDGEMENTS

We thank the volunteers who participated in the partial or whole field trials. All volunteers gave informed consent regarding the possible risks of getting tick-borne diseases during the field trials. This is a research report only. Use or mention of trade names does not constitute an official endorsement by Anastasia Mosquito Control District.

VI. REFERENCES CITED


ABSTRACT. The Talent UV light trap was compared with the CDC light trap, baited with or without dry ice, to determine the best trapping method to collect saltmarsh and freshwater mosquitoes on Anastasia Island and southern St. Johns County, Florida. Seven species of mosquitoes were collected from a saltmarsh on Anastasia Island, of which *Aedes taeniorhynchus* was the major species (90%). Seven species of mosquitoes were collected from freshwater habitats in southern St. Johns County, where *Culex quinquefasciatus* was the dominate species (81%). Overall, the CDC trap collected significantly more mosquitoes than the UV trap. For both traps, the addition of dry ice considerably increased mosquito abundance in collections.

Key Words. UV light trap, CDC light trap, dry Ice, *Aedes taeniorhynchus*, *Culex quinquefasciatus*

Insect suction traps, with and without attractants, are major tools used for surveillance of adult mosquito populations (Kline 2006). Evaluation of new traps and attractants for use within operational mosquito surveillance programs continue to be an active area of research in the field of medical entomology.

Mosquitoes perceive ultraviolet light (UV) (Allan 1994) and traps have been evaluated that use this wavelength for illumination (e.g. Allan 1994, Li et al. 2015). The Talent UV trap has been developed for the surveillance of adult mosquitoes but has not been evaluated for the collection of saltmarsh mosquitoes. The objective of the study was to compare the Talent UV light trap with the gold standard CDC light trap in order to determine the best trapping method to collect saltmarsh and freshwater mosquitoes in Anastasia Mosquito Control District.

Six Talent UV traps (model MSTRS), modified from a standard CDC light trap, were supplied by J. W. Zhu (Iowa State University, Ames, Iowa) and compared with 6 standard CDC light traps (John W. Hock Company, Gainesville, FL). The Talent UV traps were modified by adding a 190 mm (L) × 63 mm (W) × 32 mm (H) UV light bar that produced a light wavelength of 325 nm. Both traps were placed in a random complete block design separated by 30 meters in a residential community on Anastasia Island, St. Augustine, FL for the collection of saltmarsh mosquitoes. The same experimental design was used to evaluate the collection of freshwater mosquitoes in a companion study in the southern part of St. Johns County. All traps were operated 4 nights per week over a period of three weeks. Each trap was randomly assigned a location during each trap night. During the first field trial, Talent traps without dry ice were compared with dry ice-baited CDC light traps (Table 1). During the second field trial, Talent traps were baited
with dry ice while the CDC traps were operated without dry ice. In the third field trial, Talent traps and CDC traps were each operated without dry ice. One gallon Igloo containers purchased from local suppliers were used to dispense the dry ice from one pound blocks. Traps were placed 1.5 meters above ground using a Shepard’s hook.

Trap data collected from saltmarsh and freshwater areas were separately analyzed using non-parametric Kruskal-Wallis $\chi^2$ tests for each trap type and attractant combination. Differences were determined at $P < 0.05$.

A total of seven mosquito species were collected from the saltmarsh habitat. The species composition (and percent abundance in total collection) were: *Aedes taeniorhynchus* (Weidemann) (90%), *Anopheles crun- cians* Weidemann (7%), and *Culex nigripalpus* Theobald (2%) with *Ae. sollicitans* (Walker), *Cx. erraticus* (Dyar and Knab), *Ae. triseriatus* (Say), and *Masonia dyari* Belkin, Hinemann, and Page making up the remainder of the collection. Seven species of mosquitoes were collected from freshwater habitats including *Cx. quinquefasciatus* Say (81%), *An. crucians* (12%), *Ae. atlanticus* (Dyar and Knab) (3%), and *Psorophora ferox* (von Humbolt) (3%) with the remainder of the collection represented by *Ae. triseriatus*, *Culiseta melanura* (Coquillett), and *Coquillettidia perturbans* (Walker).

In the freshwater habitat, mean abundance of mosquitoes (trap/night) from both traps were not significantly different in the absence of dry ice (Table 1). But unbaited CDC light traps collected significantly more mosquitoes than unbaited Talent traps in the saltmarsh habitat. We also found that the mean number of mosquitoes from CDC traps baited with dry ice were significantly more than similar baited Talent traps. Regardless of what habitat they were collected in.

In our study, both light traps collected mosquitoes equally without the addition of dry ice from the freshwater habitat but this relationship changed considerably (i.e. 2-fold increase) when dry ice was added. It is well known that carbon dioxide is an attractant that considerably increases adult mosquito abundance in CDC light trap collections (Newhouse et al. 1966, Kline 2006). We also found that the CDC light trap outperformed the Talent trap in the saltmarsh habitat, regardless if both traps were baited with dry ice. These results are similar to what Li et al. (2015) found in Elkton, St. Johns County, FL. In summary, the CDC light trap currently remains the best adult mosquito surveillance trap available for the species we collected in our study.

We thank Catherine Lippi for assisting in data analysis. This is a research report only and does not reflect the endorsement of the Anastasia Mosquito Control District for any of the products mentioned in this study.

### REFERENCES CITED


EFFECTS OF LEAF WASHING ON THE PERSISTENCE OF A SUGAR BAIT-PYRIPROXYFEN MIXTURE TO CONTROL LARVAL Aedes albopictus

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ABSTRACT. The effect of adding the insect growth regulator, pyriproxyfen, to an attractive sugar bait (ASB) was evaluated in semi-field bioassays against larval Aedes albopictus. Treatments were applied to croton “Petra” potted plants while control plants were sprayed with ASB only. Once a week, for six weeks, plants were “washed” with water to simulate rainfall and the runoff collected for adult emergence inhibition bioassays. Adult inhibition in the ASB/pyriproxyfen treatment was about 90% for the first two weeks of the study then gradually decreased from about 80% in week three to about 30% at week six.

Key Words. Aedes albopictus, pyriproxyfen, attractive sugar bait, larvicide, croton “Petra”

Previous studies have shown that attractive toxic sugar baits (ATSB) sprayed on plants will kill adult Aedes, Anopheles, and Culex species (Xue et al. 2006, 2011, 2013, Muller et al. 2010, Naranjo et al. 2013, Revay et al. 2013, Hossain et al. 2014). Attractive toxic sugar bait is a highly effective method of controlling adult mosquitoes by exploiting their sugar feeding behaviors. Moreover, this technique has been proven to be safe for non-target insects when applied to non-flowering plants or incorporated in bait stations (Qualls et al. 2012, Revay et al. 2013). However, rainfall currently limits the effectiveness of ATSBs as barrier treatments against adult mosquitoes. In order to turn this limitation into an advantage, we report here on the addition of pyriproxyfen to a commercial attractive sugar bait (ASB) formulation to order to control Aedes albopictus (Skuse) larvae. Pyriproxyfen, by itself, is an effective larvicide that inhibits adult emergence according to laboratory bioassays reported by Ali et al. (1995). This active ingredient has also provided effective larval control of Ae. albopictus when applied by truck-mounted equipment ULV equipment in northeastern Florida (Scott et al. 2013 and Doud et al. 2014). A recent laboratory study by Fulcher et al. (2014) reported effective control of adult and larval mosquitoes from plants sprayed with an ASB and pyriproxyfen mixture. However, it was unclear whether natural precipitation on ASB pyriproxyfen-treated plants would affect the efficacy and persistence of Ae. albopictus larval control. Therefore, the objective of this study was to

determine the impact of leaf washing on efficacy and persistence of ASB/pyriproxyfen applications on plants for larval *Ae. albopictus* in a semi-field trial.

Eleven, 25 cm dia. croton, *Codiaeum variegatum* “Petra” (L.) Rumph ex. A. Juss, plants were purchased from Home Depot, St. Augustine, FL. Plants were washed off and allowed to dry before application to the leaves. A commercially available attractive sugar bait concentrate was mixed with water 1:4 ratio (Westham Innovations LTD, Tel Aviv, Israel) to which 1% NyGuard® ED (10% pyriproxyfen AI, provided by Navy Entomology Center of Excellence) was then added to the bait solution. The mixture was applied to nine plants using a 5.6 L pump-up sprayer (ACE half-gallon polypropylene sprayer) in sweeping motions over the plants until complete coverage occurred but not to the point of runoff. This technique simulated barrier applications used in field environments. Two control plants were sprayed with ASB only in the same manner as treated plants. Only one ATSB (and ASB for control) application was made to plants during the study. After a 24 hour drying period, 200ml of reverse osmosis water, (GE SmartWater™ Reverse Osmosis Filtration System, Fairfield, CN) was applied by a handheld sprayer over the surfaces of the leaves while clock-wise rotating the plants to simulate rainfall. The runoff was collected into an 11.4-L (40 cm × 31.5 cm × 15.2 cm) plastic dish pan (Sterilite Corporation, Townsend, MA) and transferred to 946 ml all-purpose-calibrated (APC) plastic containers (Ace Hardware, St. Augustine, FL). Plant washes were conducted once a week for six weeks.

*Aedes albopictus* larvae, maintained in the Anastasia Mosquito Control District, were used in bioassays. This insecticide-susceptible laboratory colony was originally obtained from the USDA, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida. Mosquito larvae were reared at 27° C; 80% RH; 12:12 L:D photoperiod and used the procedures described by Gerberg et al (1994). Ten late third to early fourth instars were placed into each APC plastic container containing runoff water and maintained in the insectary. Larvae were fed every three days with 0.03 g ground dog biscuits. Emergence in each dish pan was monitored daily until all larvae/pupae had died or emerged as adults. Experiments were repeated three times. If adult mortality exceeded 30% in control group that test was either excluded or repeated. Percent emergence inhibition was calculated as described in Scott et al. (2013).

Mean percent adult emergence inhibition values were pooled for the nine treatment plants and two control plants. Data were then arcsine square root transformed to fit the basic assumptions of ANOVA in JMP version 9 statistical software prior to analysis. Student’s paired *t*-tests were used to determine differences in treatments for each week compared with controls (*P* > 0.05).

Adult inhibition in the ASB/pyriproxyfen treatment was about 90% for the first two weeks of the study. After this time, efficacy gradually decreased to about 80% at week 3 then about 50% during weeks 4 and 5 with 30% adult inhibition at week 6.

Our data suggested that runoff from pyriproxyfen/ASB treated vegetation can provide about 90% adult inhibition of *Ae. albopictus* for at least 2 weeks after application. The results from this study may serve as a future model for individuals who want to combine sugar baits with IGRs for operational larval control in areas where barrier applications may also be useful for adult mosquito control.

The authors would like to thank Mike Smith for technical help. This is a research report only and the mention of any products does not imply Anastasia Mosquito Control District endorsement.

**REFERENCES CITED**


Fulcher, A., J. M. Scott, W. A. Qualls, G. C. Muller, and R. D. Xue. 2014. Attractive toxic sugar baits mixed


ABSTRACT. The effectiveness of separate area-wide ground thermal fog applications of DUET® (sumithrin/prallethrin) and barrier spraying of vegetation with Talstar® (bifenthrin) to control adult Aedes albopictus populations was compared at a large residential property with dense vegetation and multiple larval developmental sites in St. Augustine, Florida. The thermal fog treatment completely eliminated this mosquito pest through one week post treatment from a pretreatment landing count that averaged 24/person/3 min. Prior to the barrier treatment, the average landing rate count was 28/person/3 min. After application, landing rate counts were reduced to zero through three weeks.

Key Words. Aedes albopictus, thermal fog, barrier spray, sumithrin, prallethrin, bifenthrin

In 1985, the Asian tiger mosquito, Aedes albopictus (Skuse) became established in Houston, Texas after arriving in the USA, as eggs, in automobile tires imported from Japan (Benedict et al. 2007). Since then, this mosquito has rapidly spread throughout the southeastern parts of the USA, including Florida, and has become the most abundant and invasive container-inhabiting mosquito in the State (Lounibos et al. 2001). According to the Centers for Disease Control and Prevention, Ae. albopictus is a known vector of multiple arboviruses including chikungunya (CHIKV). In 2014, the Department of Health confirmed that Florida had 425 imported and 11 locally-acquired cases of CHIKV. Because Ae. albopictus is quite prevalent in St. Johns County, understanding and controlling this potential vector species within local residential areas is becoming increasingly important.

Prior to the 1970’s, area-wide ground thermal fogging was a mainstay and highly effective method for adult mosquito control, especially in the US. Later, this methodology was replaced in favor of ultra low volume technology due to a number of factors including a rapid spike in the cost of diesel fuel used as a carrier to deliver the pesticide. However, compared with ULV application, thermal foggers showed more consistency in droplet dispersal across distances as far as 90 m (Britch et al. 2010). Additionally, thermal fog application of pesticides provided greater mortality against the West Nile virus vector Culex quinquefasciatus Say (Britch et al. 2010). Recently, Anastasia Mosquito Control District (AMCD) has re-introduced thermal fogging into its operational adult control program (Xue et al. 2013).

During the middle 2000’s, barrier application of residual pesticides to vegetation for the purpose of controlling adult mosquitoes gained considerable interest among mosquito control programs, an interest that continues to this day (Trout et al. 2007, Qualls et al. 2013). By applying residual pesticides to mosquito harborage/resting sites (e.g. vegetation), mosquito programs can selectively target adult mosquito species such as Ae. albopictus, a daytime-biting species, at relatively
low cost (Bengoa et al. 2013). Anastasia Mosquito Control District has used insecticide barrier applications to perimeter vegetation to successfully control adult mosquitoes around golf courses and schools (Qualls et al. 2013).

The objective of our study was to compare the effectiveness of an area-wide thermal fog application of DUET® with that of a barrier application of Talstar®, against the container-inhabiting mosquito, *Ae. albopictus*, at a large local residential property.

Pre-treatment landing rate counts (LRC) were conducted by three volunteers for three minutes in three different locations approximately 35 meters apart at a 2.7 hectare County St. Johns County property (latitude 29.93049, longitude -81.343876). The property was chosen due to its numerous larval developmental sites and dense vegetation that are known habitat locations of adult *Ae. albopictus*. The study took place from 0900 to 1100 during October 2014 when peak seasonal populations of this species occur in St. Johns County.

DUET (active ingredient: 5% sumithrin® and 1% prallethrin) was applied at maximum label rate along the outside perimeter of the property and domicile using a LongRay® TS-35A thermal fogger (ADAPCO, Sanford, FL). The travel distance at which treatment was applied was 11 m per minute at a spray height of 3 meters. Wind direction and speed were monitored during application. At 30 minutes, and 24 hours after treatment, landing rate counts were conducted again for three minutes in the same locations used for pre-treatment. Landing rate counts were conducted weekly thereafter and continued until there was a significant increase in post treatment *Ae. albopictus* LRC.

Once *Ae. albopictus* LRCs rebounded after two weeks, a residual barrier application was administered, at maximum label rate, to perimeter vegetation of the property with Talstar® (active ingredient: 7.9% bifenthrin) using a Birchmeier Rec 15 backpack sprayer. The sprayer was calibrated to deliver 43.3 ounces per minute with a mixture of 3 gallons of water to 3 ounces of Talstar. The application rate was 30 meters per 3 minutes at a spray height of 3 meters. Landing rate counts were conducted 24 hours, 1 week, 2 weeks, and 3 weeks after treatment.

Mean landing rate counts were subjected to analysis using JMP® software (SAS Institute, Inc. 2007). Student’s *t*-test was used to compare pre- and post treatment LRC’s within each treatment (i.e. thermal fog and barrier).

Our results showed that there was a significant difference between LRCs pre and post for the thermal fog treatment after 24 hours (*t* = 2.15, *P* < 0.05). This application completely eliminated the *Ae. albopictus* population through 1 week post treatment from a pretreatment LRC that averaged 24/ person/3 min. For the barrier application, pre- and post LRCs were also significantly different after 24 hours (*t* = 2.15, *P* < 0.05). The pre-treatment LRC averaged 28 *Ae. albopictus*/person/3 min, then after application, LRCs were reduced to zero through 3 weeks post treatment.

Targeting an urban mosquito vector for control, like *Ae. albopictus*, can be challenging due to high population densities of the pest often produced from numerous cryptic larval habitats. Determining adult harborage sites to target control measures can be helpful in designing the scenario for barrier application. For example, one study showed that *Ae. albopictus* had high mortality in leaf bioassays after exposure to Talstar-treated wax myrtle plants (Cilek and Hallmon 2008). Plant identification can provide an indicator for harborage sites and is only one of the many variables that integrated mosquito management programs need to address when considering barrier application effectiveness. Past studies by Cilek (2008) have shown that some pyrethroid insecticides, applied as barrier treatments to vegetation, can last up to 6 weeks in controlling adult mosquitoes. Our results suggest that the barrier treatment was a better alternative for controlling *Ae.albopictus* in dense vegetation because of its longer lasting effects on the population in the field. However, we believe that by utilizing a combination of methods, such as thermal fogging and barrier spray-
ing, mosquito control districts may be able to limit costs in areas where consistent adult control is needed but may be difficult to achieve. Furthermore, AMCD will continue to judiciously use thermal fogging and barrier treatments as only one of the tools in its integrated mosquito management program in order to prevent the outbreak of mosquito-borne diseases in St. Johns County.

Special thanks go to the owners of the residence that allowed us conduct this study on their property. We are also thankful for the support of Ali Fulcher, Cat Smith and the AMCD volunteers who helped conduct the landing rate counts.

REFERENCES CITED


SAS Institute, Inc. 2007. JMP®, version 11. Cary, NC.


THE ELEVENTH ARBOVIRUS SURVEILLANCE AND MOSQUITO CONTROL WORKSHOP
SPONSORED BY AMCD AND USDA/CMAVE
ST. AUGUSTINE, FLORIDA
MARCH 25-27, 2014

PROGRAM

Tuesday (March 25, 2014)

Panel Session:

Moderator: Dr. Rui-De Xue, Director, AMCD, St. Augustine, FL

8:30 am Welcome & Introduction
Mrs. Catherine Brandhorst, Chairperson, AMCD Board of Commissioners
Dr. Ken Linthicum, Center Director, USDA/CMAVE
Mr. Neil Wilkinson, President, Florida Mosquito Control Association

8:40 am Keynote Speaker: New insights into the ecology of eastern equine encephalitis virus transmission in the southeastern USA ...
Dr. Thomas Unnasch, Distinguished USF Health Professor and Department Chair, University of South Florida, Tampa, FL

9:20 am Dengue vector control in Malaysia
Dr. Abu Ahmad, Professor, Universiti Sains, Malaysia

9:45 am The transgenic mosquitoes and its release for dengue control in Brazil
Dr. Aldo Malavasi, Director, Medfly and Mosquito Facility, Brazil

10:10 am Overview of dengue vaccine study in China
Dr. Qiang-Ming Sun, Visiting Professor, Yale University, New Haven, CT

10:35 am Mosquitoes and plants—the principle and use of plants in mosquito control
Dr. Rui-De Xue, Director, AMCD, St. Augustine, FL

10:45 am Break

Moderator: Dr. Ken Linthicum, Center Director, USDA/CMAVE, Gainesville, FL

11:00 am Real time monitoring of the dengue vector online: cost benefit of a Brazilian technology
Dr. Alvaro E. Eiras, Professor, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

10:20 am Historical perspective of dengue fever and its management control strategy in Saudi Arabia
Dr. Abdul Aziz Althbyani, Assistant Professor, University of Tabuk, Saudi Arabia

11:40 am Challenges for the malaria vector control strategies in three ecological zones (West, Central and East Africa)
Dr. Seydou Doumbia, Professor and Co-Director of MRTC/FMPOS, University of Sciences, Techniques and Technology of Bamako, Mali

12:00 pm  An update about Clarke’s new products for mosquito control  
Mr. Frank Clarke, Vice President, Clarke, Kissimmee, FL

12:10 pm  Lunch break (provided by Clarke)

Programs and Associations:
Moderator: Dr. Gary Clark, Research Leader, USDA/CMAVE, Gainesville, FL

1:00 pm  The International Center of Excellence in Research of Vector-borne Diseases in Mali  
Dr. Traore Sekou F., Professor and Co-Director of MRTC/FMPOS, University of Sciences, Techniques and Technology of Bamako, Mali

1:20 pm  Overview of USDA/CMAVE program  
Dr. Ken Linthicum, Center Director, USDA/CMAVE, Gainesville, FL

1:40 pm  USDA/Rutgers University’s area wide management program of  
(http://asiantigermosquito.rutgers.edu/)  
Dr. Gary Clark, Research Leader, USDA/CMAVE, Gainesville, FL

2:00 pm  Update on the American Mosquito Control Association  
Dr. Roxanne Connelly, President, AMCA, Professor, University of Florida/IFAS/FMEL, Vero Beach, FL

2:15 pm  Overview of the Society of Vector Ecology  
Dr. Dan. Kline, Vice President, SOVE, Research Entomologist, USDA/ARS/CMAVE, Gainesville, FL

2:30 pm  Update on the Florida Mosquito Control Association  
Mr. Neil Wilkinson, President, FMCA, Florida Gulf Coast University, Ft. Myers, FL

2:45 pm  Chemical and biological threats and vulnerabilities in industry and academia  
Mrs. Bridgette Frost, FBI Special Agent, Jacksonville, FL

3:05 pm.  Break

Arbovirus/Dengue Outbreak in Martin County, FL:  
Moderator: Dr. John Beier, Professor, University of Miami, Miami, FL

3:20 pm  Arbovirus surveillance in Florida, 2013  
Stephanie M. Moody-Geissler, Arbovirus Surveillance Coordinator, Bureau of Epidemiology, FLDOH, Tallahassee, FL

3:40 pm  Dengue outbreak in Martin County, Florida in 2013  
Mrs. Karlette Peck, Martin County Department of Health, Stuart, FL

4:00 pm  FDACS’s entomological investigation on the 2013 outbreak of dengue fever in Martin County  
Dr. Peter Jiang, Medical Entomologist, Bureau of Pesticides, FDACS, Tallahassee, FL

4:15 pm  Larval surveillance of dengue fever vectors, and in Hillsborough County, FL, 2011-2012  
Dr. Azliyati Azizan, Assistant Professor, Department of Global Health, University of South Florida, Tampa, FL
Program 101
4:35 pm Analysis of sentinel chickens and arbovirus in St. Johns County, FL
Mr. Richard Weaver, Data Manager, AMCD, St. Augustine, FL
4:45 pm WNV in Jacksonville, Florida, 2013 and activity pattern of vector mosquitoes
Ms. Marah Clark, Entomologist, Jacksonville Mosquito Control, FL
5:00 pm Cooperative projects to control in Florida and Thailand
Drs. Kenneth Linthicum and Seth C. Britch, USDA/CMAVE, Gainesville, FL
5:20 pm End of the session
6:00 pm Dinner and Lecture (Holiday Isle, Beach Blvd, St. Augustine Beach City): Highlights of the Oldest City, City Commissioner, St. Augustine, FL

Wednesday (March 26, 2014)

Moderator: Dr. Dan Kline, Research Entomologist, USDA/CMAVE, Gainesville, FL
8:30 am Sex, Y, and the control of mosquito-borne infectious diseases
Dr. Jake Tu, Professor, Virginia Tech University, Blacksburg, VA
8:50 am Gene silencing as a strategy for vector control
Dr. James Becnel and Mr. Al Estep, USDA/CMAVE, Gainesville, FL
9:10 am Update about research on carbonic anhydrases in mosquitoes
Mr. D. Dixon and Dr. Paul Linser, Professor, Whitney Laboratory, University of Florida, St. Augustine, FL
9:30 am Integrating molecular tools with Florida mosquito control
Dr. Liming Zhao, Research Assistant Professor, University of Florida/IFAS/FMEL, Vero Beach, FL
9:50 am Environmental assessment of mosquito ecology in Northern Haiti
Dr. Whitney A. Qualls, Ms D.M. Samson, and Dr. John Beier, University of Miami, FL
10:10 am Expanding integrated vector management to promote healthy environments
Dr. John Beier, Professor, University of Miami, Miami, FL
10:30 am Break

Attractants/Traps/Repellents:
Moderator: Dr. Uli. Bernier, Research Chemist, USDA/CMAVE, Gainesville, FL
10:40 am Developing new insecticides and repellents for skin and cloth
Dr. Uli. Bernier, Research Chemist, USDA/CMAVE, Gainesville, FL
11:00 am Evaluation of spatial repellents for military use
LT Marcus McDonough, Navy Entomology Center of Excellence, Jacksonville, FL
11:15 am Plant flower attraction for Aedes albopictus
Dr. Dan. Kline, Research Entomologist, USDA/CMAVE, Gainesville, FL
11:35 am Field assessment of yeast and oxalic acid generated CO₂ for mosquito surveillance
LT James Harwood, Navy Entomology Center of Excellence, Jacksonville, FL
11:50 am  Five light trap comparison for collection of mosquitoes and non-target insects  
Dr. Chun-Xiao Li, Visiting Scientist, Department of Entomology and Nematology, University of Florida, Gainesville, FL

12:00 pm  An update about ADAPCO’s new technology  
Mr. Derek Wright, ADAPCO, Sanford, FL

12:15 pm  Lunch Break (provided by ADAPCO)

Larval Habitats & Control:  
Moderator: Dr. Jerry Hogsette, Research Entomologist, USDA/ARS/CMAVE, Gainesville, FL

1:00 pm  Florida’s Indian River Lagoon: mosquito control within an estuary in distress  
Mr. Doug Carlson, Director, Indian River Mosquito Control District, Vero Beach, FL

1:20 pm  Vertical distribution of container-inhabiting mosquitoes in a LaCrosse virus endemic area  
Mr. Michael Riles, Dr. Bruce Harrison, and Dr. Brian Byrd, University of Western North Carolina, NC

1:30 pm  Acoustic techniques for mosquito control in Houston, TX  
Mr. Herbert Nyberg, New Mountain Innovations, Inc., Niantic, CT

1:50 pm  The use of cree carrying larvicides for possible control of mosquito larvae  
Mr. John Olsen, President, Cree Industries Inc., FL

2:10 pm  Aerial larviciding in the Florida Keys  
Mr. Mike Doyle, Director, Florida Keys Mosquito Control District, Key West, FL

2:30 pm  Evaluation of Mosquiron CRD against in downtown storm drains, St. Augustine  
Mrs. Ali Fulcher, Biologist, AMCD, St. Augustine, FL

2:40 pm  A durable dual action lethal ovitrap for control of container breeding mosquitoes  
Drs. Roberto Pereira and Phil Koehler, Department of Entomology and Nematology, University of Florida, Gainesville, FL

3:00 pm  Wildlife lighting- protect wildlife through responsible lighting practices  
Mr. Mike Hudon, Research Entomologist, Indian River Mosquito Control District, Vero Beach, FL

3:20 pm  Break

Adult Control:  
Moderator: Dr. Jake Tu, Professor, Virginia Tech University, Blacksburg, VA

3:40 pm  Primarily testing and formulations of an attractive targeted sugar bait against Aedes aegypti and Aedes albopictus  
Ms. Jodi Scott, Education Specialist, AMCD, St. Augustine, FL

3:50 pm  Hand thermal fogging comparison study  
Mrs. Ali Fulcher, Biologist, AMCD, St. Augustine, FL
4:00 pm  
**Sticky traps against *Aedes aegypti***  
Ms. Emily Thompson AMCD, St. Augustine, FL and Ms. Jodi Scott, Department of Entomology and Nematology, University of Florida, Gainesville, FL

4:10 pm  
**Exploring new thermal fog and ultra-low volume technologies to improve indoor control of the dengue vector,**  
LT James Harwood, Navy Entomological Center of Excellence, Jacksonville, FL

4:25 pm  
**The knight stick: a new trap for stable flies**  
Dr. Jerry Hogsette, Research Entomologist, USDA/ARS/CMAVE, Gainesville, FL

4:45 pm  
**Attractive targeted sugar baits (ATSB)**  
Mrs. Julie Lear, Marketing Director, Universal Pest Solutions, Dallas, TX

4:55 pm  
**Attractive toxic sugar bait stations for malaria vector control in Mali**  
Mr. Mohamed M. Traore, University of South Carolina, Colombia, SC

5:10 pm  
**Aerial spray to control *Aedes aegypti* in Manatee County, FL**  
Mr. Mark Latham, Director, Manatee County Mosquito Control District, FL

5:30 pm  
End of the session

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**Thursday (March 27, 2014)**

*Programs, Education, & Legislation:*

**Moderator:** Mr. Neil Wilkinson, Florida Gulf Coast University, Ft. Myers, FL

8:30 am  
**Overview of the school program at Lee County Mosquito Control District**  
Mr. Neil Wilkinson, Florida Gulf Coast University, Ft. Myers, FL

8:50 am  
**Efficacy of a new fly bait containing cyantraniliprole**  
Mr. Casey Parker, Drs. Roberto Pereira and Phil Koehler, Department of Entomology and Nematology, University of Florida, Gainesville, FL

9:00 am  
**ATSB and pyriproxyfen on plants against adult and larval *Aedes aegypti***  
Dr. Rui-De Xue, Director, AMCD, St. Augustine, FL

9:15 am  
**FLDACS update – progress on rulemaking**  
Mr. Michael Page, Chief, Bureau of Entomology and Pest Control, FLDACS, Tallahassee, FL

9:30 am  
**Overview of Mosquito Research Foundation**  
Mr. James Clauson, Director, Beach Mosquito Control District, Panama City, FL

9:45 am  
**Program overview of Volusia County Mosquito Control**  
Mr. James McNelly, Director, Volusia Mosquito Control District, New Smyrna Beach, FL

10:00 am  
**South Walton County Mosquito Control program**  
Mr. Ben Brewer, Director, South Walton County Mosquito Control District, Santa Rosa Beach FL

10:20 am  
Break
**Surveillance, Control, & Other:**
Moderator: Mr. Michael Page, Chief, Bureau of Entomology and Pest Control, FLDACS, Tallahassee, FL

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<tr>
<th>Time</th>
<th>Presentation</th>
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<tbody>
<tr>
<td>10:35 am</td>
<td>Large scale evaluation of a new barrier spray machine and barrier treatment</td>
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<td><strong>Mr. Mike Smith</strong>, Biological Technician, AMCD, St. Augustine, FL</td>
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<td>10:45 am</td>
<td>Early population outbreak of mosquitoes and control response in St.</td>
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<td><strong>Mrs. Kay Gaines</strong>, Supervisor, AMCD, St. Augustine, FL</td>
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<td>10:55 am</td>
<td>Evaluating the temporal effects of Nuvan Prostrips for filth fly control</td>
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<td><strong>LCDR Shani Gourdine</strong>, Navy Entomological Center for Excellence, Jacksonville, FL</td>
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<td>11:10 am</td>
<td>Evaluation of DeltaGard G as an acaricide</td>
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<td><strong>LT Matthew Yans</strong>, Navy Entomological Center for Excellence, Jacksonville, FL</td>
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<td>11:25 am</td>
<td>Database software with smart phone for operational mosquito control</td>
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<td><strong>Mr. Tim Morris</strong>, Mobisoft Infotech, Houston, TX</td>
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<td>11:40 am</td>
<td>Spot weather detectors &amp; recorder and possible for mosquito control operation</td>
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<td><strong>Dr. William R. Eisenstadt</strong>, Professor, Department of Electrical and Computer Engineering, University of Florida, Gainesville, FL</td>
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<td>12:00 pm</td>
<td>Update about products from UNIVAR</td>
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<td><strong>Mr. Jason E. Conrad</strong>, Industry Specialist, UNIVAR</td>
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<td>12:10 pm</td>
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END OF WORKSHOP
THE TWELFTH ARBOVIRUS SURVEILLANCE AND MOSQUITO CONTROL WORKSHOP
SPONSORED BY AMCD AND USDA/CMAVE
ST. AUGUSTINE, FLORIDA
MARCH 24-26, 2015

PROGRAM

Tuesday, March 24, 2015

Panel Session:
Moderator: Dr. Rui-De Xue, Director, AMCD, St. Augustine, FL

8:30 am Welcome & Introduction
Ms. Vivian Browning, Chairperson, AMCD Board of Commissioners
Dr. Ken Linthicum, Center Director, USDA/CMAVE, Gainesville, FL
Ms. Sue Bartlett, President of the FMCA, New Smyrna, FL

8:40 am Keynote Speaker: Epidemic of chikungunya and dengue fever and research response and focus from the University of Miami
Dr. John C. Beier, Professor and Section Chief, University of Miami, Miller School of Medicine, Miami, FL

9:20 am Guest Speaker: Overview of Rutgers University’s Center for Vector Biology
Dr. Randy Gaugler, Distinguished Professor and Director, Center for Vector Biology, Rutgers University, NJ

9:50 am Dengue virus and potential vaccine investigation
Dr. Scott Michael, Professor, Florida Gulf Coast University, Ft. Myers, FL

10:20 am The 4th International Forum for Surveillance and Control of Mosquitoes and Mosquito-borne Diseases, Guangzhou, China, (May 25-28, 2015) and AMCD’s new complex
Dr. Rui-De Xue, Director, AMCD, St. Augustine, FL

10:30 am Break

Moderator: Dr. Ken Linthicum, Center Director, USDA/CMAVE, Gainesville, FL

11:00 am Spatial Repellents for Control of Vector-borne Disease: The Current Status
Dr. Nicole Achee, Associate Professor, University of Notre Dame, IN

11:20 am Progress and prospective of attractive toxic sugar baits against mosquitoes and sand Flies
Dr. Gunter Muller, Visiting Professor, Hebrew University, Jerusalem, Israel

11:40 am Extreme weather events and impact on vector-borne diseases and agriculture
Dr. Kenneth Linthicum, Center Director, USDA/CMAVE, Gainesville, FL

12:00 pm An update on Clarke’s new products for mosquito control
Mr. Frank Clarke, Vice President, Clarke, Kissimmee, FL

12:10 pm Lunch break (provided by Clarke)
Programs & Associations:
Moderator: Dr. Dan Kline, Research Entomologist, USDA/CMAVE, Gainesville, FL
1:00 pm  ATSB in the marketplace, commercial and consumer update
Ms. Laura Uggerholt, Universal Pest Control
1:20 pm  FLDACS program update
Ms. Adriane N. Tambasco, Medical Entomologist, FLDACS/Division of Agricultural Environmental Services, Tallahassee, FL
1:40 pm  Update on the American Mosquito Control Association in 2015
Dr. Ken Linthicum, President, AMCA and Center Director, USDA/CMAVE, Gainesville, FL
1:55 pm  Overview of the Society of Vector Ecology
Dr. Dan Kline, President-Elect, SOVE and Research Entomologist, USDA/ARS/CMAVE, Gainesville, FL
2:10 pm  Update on the Florida Mosquito Control Association
Mrs. Sue Bartlett, President, FMCA and Operations Manager, Volusia Mosquito Control, New Smyrna, FL
2:25 pm  Overview of the Florida Entomological Society
Dr. Cindy McKenzie, President, Florida Entomological Society and Research Entomologist, USDA/USHRL, Ft. Pierce, FL
2:40 pm  Malaria vector mosquito’s resting behavior and movement in day time
Dr. Gunter Muller, Visiting Professor, Hebrew University, Jerusalem, Israel
3:00 pm  Break

Arbovirus:
Moderator: Dr. Randy Gaugler, Distinguished Professor and Director, Rutgers University, NJ
3:20 pm  Arbovirus surveillance in Florida, 2014: the year of chikungunya
Dr. Andrea Bingham, Arbovirus Surveillance Coordinator, Bureau of Epidemiology, FLDOH, Tallahassee, FL
3:40 pm  Long-term health impacts of chikungunya virus infection in Florida residents
Ms. Katie Kendrick, Epidemiologist, Bureau of Epidemiology, FLDOH, Tallahassee, FL
4:00 pm  West Nile virus outbreak in Volusia County, Florida in 2014
Mr. James McNelly, Director, Volusia Mosquito Control, New Smyrna, FL
4:20 pm  Vector competence of Florida mosquitoes for chikungunya and dengue viruses
Dr. Barry Alto, Assistant Professor, University of Florida/IFAS, Florida Medical Entomology Laboratory, Vero Beach, FL
4:40 pm  Arbovirus surveillance by sentinel chickens and a novel technique in St. Johns County
Miss Jennifer Gibson, Biological Technician, AMCD, St. Augustine, FL
5:00 pm  West Nile virus in Jacksonville, Florida, 2014 and daily activity pattern of vector mosquitoes
Ms. Marah Clark, Entomologist, Jacksonville Mosquito Control District, FL
5:20 pm  
End of the session

6:00 pm  
Dinner and Lecture (Holiday Isle, Beach Blvd., City of St. Augustine Beach): **Multi-rotor unmanned aerial systems: new tool for mosquito control**  
**Dr. Randy Gaugler**, Distinguished Professor and Director, Rutgers University’s Center for Vector Biology, NJ

**Wednesday, March 25, 2015**

**Biology & Ecology:**
**Moderator: Dr. Nicole Achee, Associate Professor, University of Notre Dame**

8:20 am  
**Update on the Acoustic Techniques for Mosquito Control**  
**Mr. Herbert Nyberg**, New Mountain Innovations, Inc., Niantic, CT

8:35 am  
**Mosquito gut ecosystem and potential impact on control action**  
**Dr. Jiannong Xu**, Associate Professor, Department of Biology, New Mexico State University, Las Cruces, NM

8:55 am  
**Population dynamics of the floodwater mosquito in St. Johns County, Florida**  
**Miss Emily Thomson**, Biological Technician, AMCD, St. Augustine, FL

9:05 am  
**Aedes japonicus japonicus** (Theobald): a new emerging vector in northwest Florida  
**Mr. Mike Riles**, Entomologist, Beach Mosquito Control District, Panama City Beach, FL

9:20 am  
**Update on research on carbonic anhydrases in Aedes aegypti**  
**Mr. D. Dixon** and **Dr. Paul Linser**, Whitney Laboratory, University of Florida, St. Augustine, FL

9:35 am  
**Mosquitoes and non-target insects and attractive target sugar baits**  
**Ms. Jodi Scott**, Visiting Scientist, AMCD, St. Augustine, FL and Ph.D. candidate, Department of Entomology and Nematology, University of Florida, Gainesville, FL

9:50 am  
**Mosquitoes associated with suburban backyards and dog kennels near Gainesville, FL**  
**Mr. Chris Holderman**, Ph.D. candidate, Department of Entomology and Nematology, University of Florida, Gainesville, FL

10:05 am  
**Comparison of target and non-target mortality rates from residual pesticide on HESCO material**  
**Mr. Robert L. Aldridge**, **Mr. Thomas T. Dao**, **Dr. Seth C. Britch** and **Dr. Kenneth J. Linthicum**, USDA/CMAVE, Gainesville, FL

10:20 am  
**Indoor and outdoor sugar feeding behaviors of dengue vector in Ecuador**  
**Drs. Whitney Qualls** and **John Beier**, Professor, University of Miami, Miami, FL

10:35 am  
Break

**Attractants/Traps/Repellents:**
**Moderator: Dr. James Becnel, Research Entomologist, USDA/CMAVE, Gainesville, FL**

10:40 am  
**Current research on mosquito repellents for clothing and skin**  
**Ms. Natasha Agramonte** and **Dr. Ulrich Bernier**, USDA/CMAVE, Gainesville, FL
Various approaches for the control of blood-feeding insects
Dr. Lisa L. Drake, Department of Biology, New Mexico State University, Las Cruces, NM

A multiyear study of oviposition patterns in suburban residential backyards
Dr. Dan Kline, Research Entomologist, USDA/CMAVE, Gainesville, FL

Evaluation of thermal cell mosquito repellents against several species of mosquitoes
Mr. Christopher Bibbs, Education Specialist, AMCD, St. Augustine, FL

An update on ADAPCO’s new products
Mr. Derek Wright, ADAPCO, Sanford, FL

Pesticides, Larval & Adult Control:
Moderator: Dr. Jerry Hogsette, Research Entomologist, USDA/ARS/CMAVE, Gainesville, FL

Ground application of larviciding and adulticiding at AMCD
Mrs. Kay Gaines, Supervisor, AMCD, St. Augustine, FL

New formulations of larvicide and adulticide for mosquito control ....
Mr. David Sykes, All Pro Vector Group, Madison, FL

Effects of residual IGRs on Aedes aegypti and Aedes albopictus
Ms. Kristen Donovan, Undergraduate Research Assistant, Dr. Roberto Pereira and Dr. Phil Koehler, Associate Research Scientist and Professor, Entomology Department, University of Florida, Gainesville, FL

Malaria transmission along the Niger river in a Sudan savanna area of Mali
Dr. Sekou F. Trtaore, Professor and Director of MRTC Entomology, University of Sciences, Techniques and Technology of Bamako, Mali

Program Overview: Collier Mosquito Control District
Mr. Jim Stark, Executive Director, Collier Mosquito Control District, Naples, FL

Evaluation of larvicides and pupacides against Aedes aegypti and Culex quinquefasciatus
Miss Jennifer Gibson, Biological Technician, AMCD, St. Augustine, FL

Public Health Pesticide Inventory and new insecticides
Dr. Karl Malamud-Roam, Public Health Pesticides Program Manager, IR-4 Project Headquarters, Princeton, NJ

Possible impact of mosquito control pesticides on coral reef health in Florida
Dr. Cliff Ross, Associate Professor, Department of Biology, University of North Florida, Jacksonville, FL

Evaluation of Cimi-Shield Knock-Out Bed Bug Eliminator against adult house flies
Dr. Jerry Hogsette, Lead Scientist and Research Entomologist, USDA/ARS/CMAVE, Gainesville, FL

Break
Insecticide Resistance:
Moderator: Dr. Whitney Qualls, University of Miami, Miami, FL
3:40 pm  
Resistance mechanisms in the Akron strain of *Anopheles gambiae* isolated in Benin, West Africa  

4:00 pm  
Characterization of a pyrethroid resistant strain of from Puerto Rico  
Dr. James J. Becnel, Mr. Alden Estep, Mr. Neil Sanscrainte and Ms. Jessica Louton, USDA/CMAVE, Gainesville, FL

4:20 pm  
Survey of *Aedes aegypti* in Florida to determine resistance levels and mechanisms  
Mr. Alden Estep, Dr. James J. Becnel, and Mr. Neil Sanscrainte, USDA/CMAVE, Gainesville, FL and Ms Christine Waits, Navy Entomology Center of Excellence

4:40 pm  
Comparison of pyrethroid resistance in adult and larval *Culex pipiens pallens* in five field populations from China  
Ms. Hui Liu, Research Associate, Department of Vector Biology and Control, Jiangsu Provincial CDC, Nanjing, China

5:00 pm  
Testing for insecticide resistance in *Aedes albopictus* strains from St. Augustine, Florida  
Ms. Christy Waits, Navy Entomology Center for Excellence, Jacksonville, FL, Mrs. Ali Fulcher, AMCD, Mr. Alden Estep, Mr. Alec Richardson, Navy Entomology Center for Excellence, Jacksonville, FL, Dr. Jimmy Becnel, USDA/CMAVE, and Dr. Rui-De Xue, AMCD

5:20 pm  
Vector epidemiology and challenge of malaria control in Mali  
Dr. Seydou Doumbia, Professor and Director, MRTC Entomology, University of Sciences, Techniques and Technology of Bamako, Mali

5:40 pm  
End of the session

Thursday, March 26, 2015

Technology & Program:
Moderator: Ms. Sue Bartlett, Operations Manager, Volusia Mosquito Control, New Smyrna, FL

8:30 am  
SMACK those mosquitoes: a “Sentinel Mosquito Arbovirus Capture Kit” (SMACK) for monitoring arboviruses in the field  
Dr. Scott Ritchie, Professor, James Cook University, Cairns, Australia

9:00 am  
Overwintering of eastern equine encephalitis virus in North America  
Dr. Nathan Burkett-Cadena, Assistant Professor, University of Florida/IFAS/FMEL, Vero Beach, FL

9:20 am  
Durable Dual-action Lethal Ovitraps (DDALO) for management of the dengue vector and other container-breeding mosquitoes  
Ms. Casey Parker, Graduate Research Assistant, Drs. Roberto Pereira and Phil Koehler, Associate Research Scientist and Professor, Department of Entomology and Nematology, University of Florida, Gainesville, FL

9:35 am  
Overview of the IGR-pyriproxyfen through auto-dissemination against dengue vector mosquitoes  
Mr. Mike Banfield, CEO, Spring Star, Inc. Seattle, WA
9:50 am Database software with smart phone for operational mosquito control in AMCD
Mr. Richard Weaver, Data Manager, AMCD and Mr. Tim Morris, Mobi-soft Infotech, Houston, TX

10:05 am GeoWorld Outdoors, Inc. GeoMosquito Mapping Solution
Mr. Daniel Finch, GeoWorld Outdoors, Inc, Kenosha, WI

10:20 am Mobile application with a focus on pest activity, material target pests, and e-content
Mr. Adam Holt, Service Pro, St. Augustine, FL

10:30 am Break

Surveillance & Other:
Moderator: Dr. Nathan Burkett-Cadena, Assistant Professor, UF/IFAS/FMEL, Vero Beach

10:50 am Population surveillance of Aedes albopictus detected by BGS traps in St. Johns County, FL
Mr. Mike Smith, Biologist, AMCD, St. Augustine, FL

11:00 am Development of a rapid detection method for arbovirus surveillance in Florida
Dr. Limin Zhao, Research Assistant Professor, University of Florida/IFAS/FMEL, Vero Beach, FL

11:20 am Understanding IGRs and their potential for controlling bed bugs
Ms. Brittany Delong, Graduate Research Assistant, Drs. Roberto Pereira and Phil Koehler, Associate Research Scientist and Professor, Department of Entomology and Nematology, University of Florida, Gainesville, FL

11:40 am Mosquito population surveillance and GIS
Dr. Mohamed F. Sallam, Visiting Scientist, Department of Entomology and Nematology, University of Florida, Gainesville, FL

12:00 pm Intact Immunoglobulin traffic from blood meal to hemoplymph: a new look at mosquito control via host immunization
Dr. Paul Linser, Professor, Whitney Laboratory, University of Florida, St. Augustine, FL

12:20 pm Update on products from UNIVAR
Mr. Jason E. Conrad, Industry Specialist, UNIVAR

12:30 pm Lunch break (provided by UNIVAR)

END OF WORKSHOP

2:30 pm New AMCD facility ground breaking