FIELD STUDIES AND MONITORING OF MOSQUITO POPULATIONS (DIPTERA:

CULICIDAE) IN URBAN ENVIRONMENTS

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information.

Whitney Allyn Qualls

Certificate of Approval:

George W. Folkerts Professor Biology

Wayne E. Clark Professor Entomology and Plant Pathology Gary R. Mullen, Chair Professor Entomology and Plant Pathology

Stephen L. McFarland Acting Dean Graduate School

FIELD STUDIES AND MONITORING OF MOSQUITO POPULATIONS (DIPTERA: CULICIDAE) IN URBAN ENVIRONMENTS

Whitney Allyn Qualls

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Degree of

Master of Science

Auburn, Alabama December 16, 2005

FIELD STUDIES AND MONITORING OF MOSQUITO POPULATIONS (DIPTERA: CULICIDAE) IN URBAN ENVIRONMENTS

Whitney Allyn Qualls

Permission is granted to Auburn University to make copies of this thesis at its discretion, upon request of individuals or institutions and at their expense. The author reserves all publication rights.

Signature of Author

Date

VITA

Whitney Allyn Qualls, daughter of Jerry Allen Qualls and Sara Jo (Costner) Qualls, was born December 31, 1980, in Athens, Tennessee. She graduated from McMinn Central High school in 1999. She attended Cumberland College in Williamsburg, Kentucky, graduating in 2003 with a Bachelor of Science Degree in Biology. In 2003 she entered the Master of Science program in the Department of Entomology and Plant Pathology at Auburn University.

THESIS ABSTRACT

FIELD STUDIES AND MONITORING OF MOSQUITO POPULATIONS (DIPTERA: CULICIDAE) IN URBAN ENVIRONMENTS

Whitney Allyn Qualls

Master of Science, December 16, 2005 (B.S., Cumberland College, 2003)

97 Typed Pages

Directed by Gary R. Mullen

In 2004 and 2005 field work was conducted to survey and monitor larval and adult mosquito populations in urban habitats with, primary interest in *Aedes albopictus*, the Asian tiger mosquito.

In 2004 and 2005 a state-wide survey of tire-breeding mosquitoes was conducted. Tire sites in all 67 counties in the state of Alabama were sampled for mosquito larvae. A total of 13,022 mosquito larvae, representing 13 mosquito species in 7 genera, was collected. The most frequently collected species were *Ae. albopictus* (71%),*Culex territans* (7.5%), and *Ochlerotatus triseriatus* (7.1%). The following species were also collected: *Cx. restuans* (5.2%), *Cx. salinarius* (3.5%), *Orthopodomyia signifera* (2.7%), *Cx. quinquefasciatus* (1.2%), *Oc. atropalpus* (<1%), *Toxorhynchites rutilus* (<1%) Anopheles punctipennis (<1%), An. quadrimaculatus (<1%), An. spp. (<1%), and Psorophora columbiae (<1%). No Ae. aegypti or Oc. japonicus were collected from tires during this survey. Psorophora columbiae was also collected from discarded tires, representing the first recorded collection of this species from tires.

This study demonstrated that since the first detection of *Ae. albopictus* at Cullman, Alabama in 1985, *Ae. albopictus* has established itself throughout the state becoming the most common tire-inhabiting mosquito in Alabama. Results also showed that the yellow fever mosquito *Ae. aegypti* is no longer the dominant tire-breeder in Alabama. In fact it appears that *Ae. aegypti* has been displaced from tires throughout state.

In 2004, studies were conducted to evaluate the short-range mosquito attractant 1octeno-3-ol (octenol) used with commercially available propane-powered mosquito traps to increase collections of urban mosquitoes. Octenol was evaluated using the Mosquito Magnet Pro^{TM} (MMP) trap. Three field trials were conducted in the communities of Auburn and Phenix City, Alabama. Four MMP traps were placed in a 1x2 factorial design. *Aedes albopictus, Coquillettida perturbans*, and *Oc. triseriatus* collections were significantly enhanced with octenol as determined with a 3-way ANOVA, P < .05. *Anopheles punctipennis, Ps. columbiae, Cx. restuans,* and *Cx. salinarius* collections were also significantly enhanced with octenol as determined by a Chi-square analysis, P < .05. Twelve out of 13 mosquito species were collected in greater numbers with octenol than without octenol throughout this study.

ACKNOWLEDGEMENTS

The author would like to thank her advisor Gary R. Mullen, her committee members George W. Folkerts and Wayne E. Clark; Ashley Lovell and the Alabama Department of Public Health for assistance in organizing the state-wide tire-breeding mosquito survey; and Matthew Smith, Scott Croxton, Will Sherrer and Nathan Burkett who helped in field work. She also would like to thank her parents for their love and support. Style manual or journal used: Journal of the American Mosquito Control Association

Computer software used: Microsoft Word $\ensuremath{\mathbb{R}}$

TABLE OF CONTENTS

LIST OF TABLES	xi
LIST OF FIGURES	xiv
I. INTRODUCTION	1
Literature Cited	4

II. STATE-WIDE SURVEY OF TIRE-BREEDING MOSQUITOES

(DIPTERA: CULICIDAE) IN ALABAMA	7
Materials and Methods	11
Results	12
Discussion	14
Literature Cited	19

III. EVALUATION OF THE MOSQUITO ATTRACTANT

OCTENOL FOR COLLECTING URBAN MOSQUITOES	54
Study Sites	57
Materials and Methods	59
Results	61
Discussion	64

	Literature Cited	
APPEN	DX	

LIST OF TABLES

- Presence or absence of tire-breeding mosquitoes by 2-week intervals from May through October, based on the 2004-2005 larval survey. Species are listed in descending order of the total numbers of each species collected......40

6.	Mosquito species collected with and without octenol at Field Site 1, June 2004.	
	Species are listed in descending order of the total number of each species	
	collected	.69
7.	Repeated measures ANOVA values for mosquito species with trap collections	
	>100 specimens at Field Site 1	70
8.	Mosquito species collected with and without octenol at Field Site 2.	
	June July 2004 Species are listed in descending order of the total	
	June-July 2004. Species are listed in descending order of the total	
	number of each species collected	71
9.	Repeated measures ANOVA values for mosquito species with trap collections	
	>100 specimens at Field Site 2	72
10		
10.	Mosquito species collected with and without octenol at Field Site 3,	
	July-August, 2004. Species are listed in descending order of the total number	
	of each species collected	73
11.	ANOVA values for mosquito species with trap collections > 100 specimens at	
-	Field Site 3	74

LIST OF FIGURES

1.	Map of Alabama counties reproduced from Department of Geography,	
	College of Arts and Sciences, the University of Alabama4	4

- Map of Alabama physiographic regions reproduced from the Department of Geography, College of Arts and Sciences, the University of Alabama......45
- Alabama counties in which larvae were collected from tires, shown in gray.
 a, 2004. b,2005......46
- Map of Alabama showing number of tire sites sampled per county during the 2004-2005 larval survey. A total of 169 tire sites sampled......47

7.	Distribution, by county, of tire-breeding mosquitoes in Alabama, based on 2004-
	2005 larval survey. Psorophora columbiae, Tx. rutilus, An. punctipennis,
	and <i>An. quadrimaculatus</i>
8.	Distribution, by county, of tire-breeding mosquitoes in Alabama, based on 2004-
	2005 larval survey. <i>Anopheles</i> spp
9.	Occurrence of tire-breeding mosquitoes by 2-week intervals from May through
	October, based on the 2004-2005 larval survey. Species are listed in
	descending
10.	Map of Alabama showing the 5 physiographic regions and the number of tire sites
	sampled per county by physiographic region based on the 2004-2005 larval
	survey
11.	American Biophysics Corporation's propane-powered Mosquito Magnet Pro TM
	trap76
12.	Collections of landing mosquitoes using a hand-held, battery-operated aspirator to
	remove landing females during a 2-minute period to determine biting activity at a
	parts-and-salvage yard, in Phenix City, Alabama, 200477

I. INTRODUCTION

Tires have long been recognized as important sites for mosquito development. The ability of discarded tires to hold and maintain water allows for successful larval habitats for many mosquito species to develop. Because of this ability tire dumps promote the proliferation and dispersal of mosquitoes, increasing the potential of public health problems ranging from nuisance complaints to transmission of mosquito-borne diseases.

Baumgartner (1988) stated that the "tire problem" is magnified because tirebreeding mosquitoes develop rapidly, enhancing their vector ability. The most notable tire-breeding mosquito species of medical importance in the southeastern United States are *Aedes albopictus*, *Ae. aegypti*, *Ochlerotatus triseriatus*, and *Oc. japonicus*. Each of these species is a competent vector for more than one important disease of humans.

Aedes albopictus, an Asian species introduced into the continental U.S. in 1985 (Francy et al. 1990) via tire shipments from Asia (Spenger and Wuithiranyagool 1986), has established itself in the eastern half of the U. S. (Moore and Mitchell 1997, Moore 1999). *Aedes albopictus* is a competent vector of Dengue and Yellow Fever Viruses (Gokhale et al. 2001). Laboratory tests have shown that *Ae*. *albopictus* is a competent vector of over 22 arboviruses (Moore and Mitchell 1997) including Ross River Virus and West Nile Virus (Mitchell et al. 1987).

Eastern Equine Encephalitis Virus (Moore and Mitchell 1997) and La Crosse Virus (Erwin et al. 2002) has been isolated from field-collected specimens. This mosquito breeds in a variety of natural and artificial containers.

Aedes aegypti, introduced into the Americas via the slave trade, is the primary vector of Yellow Fever Virus (Fontenille et al. 1997). This species is also a competent vector of Dengue Virus (Thavara et al. 2001). In the 1960s, *Ae. aegypti* dispersal in the continental United States was linked to interstate tire shipments (Haverfield and Hoffman 1966). This mosquito breeds in a variety of natural and artificial containers.

Ochlerotatus triseriatus, the primary vector of La Crosse Encephalitis Virus in the upper Midwest (DeFoliart et al. 1986), is widely distributed across the eastern half of the U.S. and southern Canada (Darsie and Ward 1981). The number of La Crosse cases has been directly linked to the number of discarded tries containing *Oc. triseriatus* (Beier et al. 1982). This species breeds primarily in water-filled cavities in deciduous trees, but also in artificial containers such as tires (Joy et al. 2003).

Ochlerotatus japonicus, a native of Japan and Korea (Tanaka et al. 1979), was first detected in America in New York in 1998 (Peyton et al. 1999). Peyton et al (1999) suggested that the mode of introduction into the northeastern United States was in used tires. This mosquito has recently extended its southern range into Georgia, North Carolina, and Alabama (Gray et al. 2005 and Mullen 2005). *Ochlerotatus japonicus* is the primary vector of Japanese Encephalitis Virus in its native range. This species is an efficient laboratory vector of West Nile Virus (Sardelis and Turell 2001), La Crosse Virus (Sardelis et al. 2002), and Eastern Equine Encephalitis Virus (Sardelis et al. 2002). West Nile Virus has been detected in multiple pools of *Oc. japonicus* collected in the

northeastern United States during the summer of 2000 (Centers for Disease Control and Prevention 2000). This species is a container-breeder, utilizing both natural and artificial containers (Scott et al. 2001).

Since many medically important mosquito species utilize tires as larval habitats, it is important to know which mosquitoes are breeding in tires in urban settings. One way to determine the mosquito species breeding in tires is by sampling tires for mosquito larvae. Another method involves collecting adults using various trapping methods and attractants.

Many tire-breeding mosquitoes are difficult to capture using standard mosquito trapping devices such as the CDC light trap and gravid trap. In recent years octenol, a chemical widely used to attract blood-feeding insects, has been used to enhance mosquito collections. Octenol has not been evaluated for enhancing collections of tire-breeding mosquitoes. The response of *Ae. albopictus* to octenol is of particular interest because of its large populations in urban areas and its vectorial capacity for mosquito-borne viruses.

In 2004 and 2005 field work was conducted in Alabama to survey and monitor tirebreeding mosquito populations in urban settings. Discarded-tire sites were sampled to determine species breeding in tire in Alabama, species distribution by county, the extent of *Ae. aegypti* displacement in tires, the seasonal occurrence of tire-breeding mosquitoes in Alabama, and the correlation of seasonal occurrence of mosquitoes by physiographic region in Alabama. Adult populations were also monitored at three field sties at Auburn and Phenix City, Alabama to determine if octenol enhances collections of tire-breeding mosquitoes.

Literature Cited

- Baumgartner DL. 1988. Suburban accumulations of discarded tires in northeastern Illinois and their associated mosquitoes. *J Am Mosq Control Assoc* 4:500-508.
- Beier JC, Travis M, Patricoski C, Kranzfelder J. 1983. Habitat segregation among larval mosquitoes (Diptera: Culicidae) in tire yards in Indiana, USA. *J Med Entomol* 20:76-80.
- Centers for Disease Control and Prevention. 2000. Update: West Nile virus activityeastern United States, 2000. *MMWR* 49:1044-1047.
- Darsie RF Jr, Ward RA. 1981. *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*. Salt Lake City, UT: American Mosquito Control Association.
- DeFoliart GR, Watts DM, Grimstad PR. 1986. Changing patterns of mosquito-borne arboviruses. J Am Mosq Control Assoc 2:437-455.
- Erwin PC, Jones TF, Gerhardt RR, Halford SK, Smith AB, Patterson LER, Gottfried KL, Burkhalter KL, Nasci RS, Schaffner W. 2002. La Crosse encephalitis in Eastern Tennessee: clinical, environmental, and entomological characteristics from a blinded cohort study. *Am J of Epidemiology* 155:1060-1065.
- Fontenille DL, Lochouarn ND, Sokhna C, Lemasson JJ. 1997. High annual and seasonal variations in malaria transmission by anophelines and vector species composition in Dielmo, a holoendemic area in Senegal. *Am J Trop med Hyg* 56:247-253.
- Francy DB, Karabatsos N, Wesson DM, Moore CG, Lazuick JS Jr, Niebylski ML, Tsai TF, Craig GB Jr. 1990. A new arbovirus from *Aedes albopictus*, an Asian mosquito established in the United States. *Science* 250:1738-1740.

- Grimstad PR, Haramis LD. 1984. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus III: Enhanced oral transmission by nutrition deprived mosquitoes. *J Med Entomol* 21:249-256.
- Joy JE, Hanna AA, Kennedy BA. 2003. Spatial and temporal variation in the mosquitoes (Diptera: Culicidae) inhabiting waste tires in Nicholas County, West Virginia. J Med Entomol 40:73-77.
- Mitchell CJ, Miller BR, Gubler DJ. 1987. Vector competence of *Aedes albopictus* from Houston, Texas, for dengue serotypes 1 to 4, Yellow Fever and Ross River viruses. *J Am Mosq Control Assoc* 3:460-465.
- Moore CG. 1999 *Aedes albopictus* in the United States: current status and prospects for further spread. *J Am Mosq Control Assoc* 15:221-227.
- Moore CG, Mitchell CJ. 1997. *Aedes albopictus* in the United States: ten-year presence and public health implications. *Emerg Infec Dis* 3:329-334.
- Mullen GR. 2005. First report of Ochlerotatus japonicus in Alabama. Alabama Vector Management Society Newsletter 15 (2):2.
- Peyton EL, Campbell SR, Candeletti TM, Romanowski M, Crans JW. 1999. Aedes (Finlaya) japonicus japonicus (Theobald), a new introduction into the United States. JAm Mosq Control Assoc 15:238-241.
- Sardelis MR, Dohm DJ, Pagac B, Andre RG, Turell MJ. 2002. Experimental transmission of Eastern Equine Encephalitis virus by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *J Med Entomol* 39:480-484.
- Sardelis MR, Turell MJ. 2001. Ochlerotatus j. japonicus in Frederick County, Maryland: discovery, distribution, and vector competence for West Nile virus. J Am Mosq Control Assoc 17:137-141.
- Scott JJ, Carle FL, Crans JW. 2001. *Ochlerotatus japonicus* collected from natural rockpools in New Jersey. *J Am Mosq Control Assoc* 17:91-92.

- Sprenger D, Wuithiranyagool T. 1986. The discovery and distribution of *Aedes* albopictus in Harris County, Texas. J Am Mosq Control Assoc 2:217-218.
- Tanaka K, Mizusawa K, Saugstad ES. 1979. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). *Contrib Am Entomol Inst* (Ann Arbor) 16: 1-987.
- Thavara U, Tawtsin A, Chansang C, Kong-ngamsuk W, Paosriwong S, Boon-Long J, Rongsriyam Y, Komalamisra N. 2001. Larval occurrence, oviposition behavior and biting activity of potential mosquito vectors of dengue on Samui Island, Thailand. J Vec Ecology 26:172-180.
- Thompson WH, Anslow RO, Hanson RP, DeFoliart GR. 1972. La Crosse virus isolations from mosquitoes in Wisconsin, 1964-1968. *Am J Trop Med Hyg* 23:694-700.

II. STATE-WIDE SURVEY OF TIRE-BREEDING MOSQUITOES (DIPTERA: CULICIDAE) IN ALABAMA

Objectives

- 1. To determine the species of mosquitoes breeding in tires in Alabama.
- 2. To determine the species distribution by county throughout the State.
- 3. To determine the extent to which *Aedes albopictus* has displaced *Aedes aegypti* as the dominant tire-breeding mosquito in Alabama.
- 4. To determine the seasonal occurrence of mosquitoes breeding in tires in Alabama.
- 5. To determine if there is a correlation between seasonal occurrence of tire-breeding mosquitoes and the physiographic regions of Alabama.

In order to effectively apply mosquito-control measures, the diversity of mosquito larval habitats must be understood. Tires are just one of many larval development habitats that provide sufficient nutrients that allow mosquitoes to flourish. Over the years studies have focused on tires as mosquito larval habitats.

The importation of mosquito larvae in tires was first reported in the mid 1940s. Pratt et al. (1946) reported that shiploads of tires arriving from Asian ports were heavily infested with seven mosquito species including *Ae. albopictus*. Haverfield and Hoffman (1966) investigated intrastate movement of tire shipments in Texas and associated that movement with the dispersal of *Ae. aegypti* across the state. Other studies, including Beier et al. (1983), focused on identifying the ecological factors that regulate mosquito production in tires.

Studies have also investigated the likelihood that natural container-breeding mosquitoes, such as *Ochlerotatus triseriatus* and *Oc. atropalpus*, would oviposit in tires (Haramis 1984, Restifo and Lanzaro 1980, Berry and Craig 1984). These studies concluded that both *Oc. triseriatus* and *Oc. atropalpus* readily oviposit in tires, potentially altering their development time and their vectorial capacity (Haramis 1984, Baumgartner 1988). However, after the introduction and establishment of *Ae. albopictus* in the United States (1985) via the tire trade (Sprenger and Wuithiranyagool 1986), attention has shifted to recognizing the importance of tires as a means of introduction of nonindigenous mosquito species. Reiter and Sprenger (1987) investigated the tire trade and found that just after the initial discovery of *Ae. albopictus* in Texas, 12 other states were infested with *Ae. albopictus* populations. Nearly all of the infestations were in tires.

With the ever increasing human population and accumulation of used tires in urban areas, along with the introduction of *Ae. albopictus*, focus has again shifted to recognizing the importance of discarded tires as a major source of mosquito production. Many studies have been conducted to determine what mosquito species are breeding in tires. A study in Connecticut (1988) investigated nine tire disposal sites to determine the mosquito species present with specific interest in *Ae. albopictus* (Andreadis 1987). *Aedes* *albopictus* at that time had not yet extended its distribution into Connecticut, but had been found in surrounding states Maryland and Delaware (Centers for Disease Control and Prevention 1987). Another study in Illinois investigated the species composition and abundance of mosquito larvae at a large tire dump site where *Ae. albopictus* had previously been reported (Lampman et al. 1997). Other studies have focused on characterizing tires habitats, to determine factors influencing mosquito production (Morris and Robinson 1994, Joy Hildreth-Whitehair 2000, Joy et al. 2003). From these studies 18 mosquito species have been reportedly collected from tires in the eastern half of the United States. These species are *Ae. albopictus, Ae. aegypti, Ae. vexans, Anopheles barberi, An. punctipennis, An. quadrimaculatus, Culex pipiens, Cx. quinquefasciatus, Cx. restuans, Cx. salinarius, Cx. territans, Culiseta melanura, Ochlerotatus atropalpus, Oc. bahamensis, Oc. triseriatus, Oc. japonicus, Orthopodomyia signifera,* and *Toxorhynchites rutilus.* See Appendix for a list of the mosquito species and their respective authors mentioned throughout the text.

Sixteen of the above 18 mosquito species occur in Alabama, the exceptions being *Cx. pipiens* and *Oc. bahamensis* (Darsie and Ward 2005). *Culex pipiens* is widely distributed across the northern United States and in British Columbia, Canada. *Ochlerotatus bahamensis* distribution is localized to only the southeastern portion of Florida.

The first detection of *Ae. albopictus* in the state of Alabama was from a tire site in Cullman County in 1986. There has never been a survey of tire-breeding mosquitoes in Alabama prior to, and since, the introduction of *Ae. albopictus*. This survey was conducted to determine what mosquito species are breeding in tires in Alabama. *Aedes*

aegypti was once the dominant mosquito breeding in tree holes and artificial containers, including tires in Alabama. Recent studies have suggested changes in species composition and dominance structure (Edgerly et al. 1993) since the establishment of *Ae. albopictus* in North America. *Aedes albopictus* has been associated with the displacement of native container-breeding mosquitoes, such as *Ae. aegypti* and *Oc. triseriatus* (Juliano 1998). In the last 17 years, *Ae. aegypti* has been scarce or absent in Alabama (G. R. Mullen unpublished). One objective of this survey was to determine to the abundance of *Ae. aegypti* in tires in Alabama.

This is one of the few comprehensive state-wide surveys of tire-breeding mosquitoes that has been conducted in the United States. In Florida *Ae. albopictus* movement has been documented since its first detection in 1986. In 1992 *Ae. albopictus* was collected in 64 out of 67 counties in Florida from containers such as tires and cemetery vases (O'Meara et al. 1993) Another comprehensive study was conducted in West Virginia over a six-year period. Containers, including tires, from 54 of 55 West Virginian counties were sampled for container-breeding mosquitoes (Joy and Hildreth-Whitehair 2000). These data were correlated with the population size of Oc. *triseriatus* and the incidence of La Crosse Virus in the counties sampled.

Seasonal data of tire-breeding mosquitoes generated by this survey will be useful for mosquito-control programs throughout the State and in determining if there is any correlation between the seasonal occurrence of tire-breeding mosquitoes and the different physiographic regions of Alabama. Because temperature directly affects developmental time, it is possible that seasonal differences in the emergence of tire-breeding mosquitoes may occur in these five physiographic regions.

Materials and Methods

This study was organized by coordinating the Alabama Department of Public Health area administrators and county environmentalists to sample discarded tires in their respective counties from May 1 to October 31, 2004 (Figure 1). This was followed by sampling tires in 2005 in counties in which tire-breeding mosquitoes were not collected during the summer of 2004. The tire sites sampled ranged from two tires per tire site to huge tire sites with over 50,000 tires. In 2004 two or more tire sites per county were sampled twice a month, one collection taken during the first half of the month and the second collection during the latter half of the month. One or two tire sites were sampled from the remaining counties during 2005.

Each ACHD county environmentalist was asked to follow the same sampling protocol: (1) agitate the tire before collecting a sample to ensure that both bottom-feeding and top-feeding larvae were sampled, (2) collect a sample using either a dipper or baster, (3) filter the sample through a small aquarium net, (4) invert the aquarium net into a small container of alcohol, and (5) pipette the larvae from the small container of alcohol into a four-dram vial of 70% ethyl alcohol. At least four tires were sampled from each tire site, with a minimum collection of 20 larvae per collection site.

Larvae were sent to the medical entomology lab at Auburn University via the Alabama Department of Public Health Courier system to be identified. All third- and fourth-instar larvae were identified using Darsie and Ward's (1981) *Identification and* *Geographical Distribution of the Mosquitoes of North America, North of Mexico*. Firstinstar larvae, second-instar larvae, and pupae were not identified during this survey. Voucher specimens for each species have been deposited in the Auburn University Insect Collection.

The data generated from this survey were used to determine if there was a correlation between the physiographic regions in Alabama (Figure 2) and seasonal occurrence of tire-breeding mosquitoes in these regions. The five physiographic regions are: Highland Rim, Cumberland Plateau, Alabama Valley and Ridge, Piedmont Upland, and the East Gulf Coastal Plain. Soils in the Highland Rim region are mostly red clay of limestone origin. The forests are comprised mostly of hardwoods, red cedar, and some pines. The Cumberland Plateau has mostly sandy loams, although clay soils are not uncommon in the southern portion of this region. The Alabama Valley and Ridge has soils ranging from gravelly loams to clay where the Piedmont Upland has clay soils that tend to be rocky. The East Gulf Coastal Plain soils vary from acid sands and sandy loams to heavy, calcareous, alkaline types. (Mount 1975)

Results

Tires were sampled in 52 of 67 counties from May 1 to October 31, 2004 (Figure 3a). The 15 counties in which collections were not made in 2004 were: Cherokee, Coosa, Houston, Jefferson, Lamar, Lawrence, Limestone, Madison, Marengo, Perry, Randolph, Tallapoosa, Walker, Wilcox, and Winston, (Figure 3b). Tires were sampled from the counties that did not collect larval samples in 2004 during the summer of 2005.

Houston County was sampled in April 2005; samples were collected from Cherokee, Jefferson, Lamar, Lawrence, Limestone, Madison, Randolph , Tallapoosa ,Walker, and Winston in July; and Coosa, Marengo, Perry, and Wilcox counties in August. County environmentalist in Jefferson and Houston Counties collected larval samples in their respective counties in 2005. The 13 remaining counties were sampled by going myself to each county and locating a tire site. A total of 169 tire sites was sampled from throughout this survey (Figure 4) with a total of 13,022 mosquito larvae identified, representing 12 mosquito species in 7 genera (Table 1).

The most frequently collected mosquito species were *Ae. albopictus* (71%), *Cx. territans* (7.5%), and *Oc. triseriatus* (7.1%). The following species were also collected: *Cx. restuans* (5.2%), *Cx. salinarius* (3.5%), *Or. signifera* (2.7%), *Cx. quinquefasciatus* (1.2%), *Oc. atropalpus* (<1%), *Tx. rutilus* (<1%), *An. punctipennis* (<1%), *An. quadrimaculatus* (<1%), *An.* spp. (<1%), and *Ps. columbiae* (<1%). Total larval collections throughout this survey by county and month are shown in Table 2. The geographic distribution, by county, of each mosquito species collected in this survey from tires sites is presented in Figures 5-8.

Aedes albopictus was collected from all 169 tire sites sampled, indicating that it is established in every county in Alabama. *Aedes albopictus* overlapped in tire yards with *Oc. triseriatus* in 39 counties in Alabama. No *Ae. aegypti* larvae were found in tires during this study, nor larvae of the recently introduced *Oc. japonicus*.

There was no difference in the seasonality of *Ae. albopictus* and *Oc. triseriatus* by 2-week intervals observed in tires during this survey (Figure 9). There was a seasonal difference observed in collections of *Oc. atropalpus*, *Ps. columbiae*, and *Anopheles*

species. *Ochlerotatus atropalpus* was collected first during the first half of June and then was not collected again until the second half of July. *Psorophora columbiae* was only collected during the last half of June and the month of July. *Anopheles* species were only collected from tires during the first part of the summer months. The remaining species were collected consistently throughout each collection period, i.e., first half or second half of months May-October, 2004 and 2005 (Table 3).

The data were not sufficient to show any correlation between physiographic region and seasonal occurrence of tire-breeding mosquitoes (Tables 4 and 5). *Anopheles* species, *Cx. quinquefasciatus, Cx. salinarius, Oc. atropalpus, Or. signifera*, and *Ps. columbiae* larvae were not collected in the Piedmont region in Alabama throughout this survey. However, distributions of these species fall within the Piedmont region according to Darsie and Ward (2005). *Anopheles quadrimaculatus, Oc. atropalpus*, and *Ps. columbiae* larvae were not collected in the Cumberland Plateau or Highland Rim regions, although the reported distributions of these species fall within these regions (Darsie and Ward 2005) (Figure 10).

Discussion

Throughout this survey tire dump sites, service stations, tire dealers, auto repair shops, and salvage yards were sampled in rural and urban areas of Alabama. All of these sites produced mosquitoes suggesting that the "tire problem" (Baumgartner 1988) is not just localized to large tire dump sites in rural areas. A total of 13 mosquito species was collected from tires in Alabama. Many of the urban tire sites sampled had more than one species of mosquito present throughout the entire survey. *Aedes albopictus* was collected from tire sites in combination with the 12 other mosquito species collected throughout the survey. *Culex territans* was frequently collected from the same tire sites as Cx. *restuans* and *Oc. triseriatus*. Although previous studies had reported that *Cx. territans* does not commonly utilize tires as a larval habitat (Wilmot et al. 1992, Jamieson et al. 1994), Joy et al. (2003) found relatively high occurrence of *Cx. territans* larvae in abandoned tire sites in their survey in West Virginia. Our data supports Joy et al.'s findings in that *Cx. territans* was the second most frequently collected tire-breeding species in Alabama throughout the survey.

The distributions of 12 of the 13 mosquito species fell within their previously known ranges (Darsie and Ward 2005). However, collections of *Oc. atropalpus* occurred farther south, in Montgomery County, than its distribution shown in Darsie and Ward (2005). Craig (1980) suggested that once *Oc. atropalpus* was introduced into tire yards by both local and interstate transportation of discarded tires, this species would probably extend its range. Since *Oc. atropalpus* females are autogenous for their first ovarian cycle (O'Meara and Krasnick 1970), they can exploit large numbers of tires, in turn extending their distribution (Beier et al 1983). *Ochlerotatus atropalpus* was collected from only one tire site (Montgomery County) on one occasion. This does not necessarily confirm that *Oc. atropalpus* has extended its southern range in Alabama as this sample could have been collected from a tire recently shipped from an area where *Oc. atropalpus* populations are known to occur.

The collections throughout this survey were inconsistent. Many counties did not collect from more than one tire site. Other counties only sampled during one month of the survey period. On the other hand, some counties were very consistent with their larval collections, collecting from more than one tire site throughout the survey. Because of this no conclusions on the seasonality of tire-breeding mosquitoes in Alabama can be made from the data obtained.

Psorophora columbiae was collected from only one tire site (Pickens County) in June and July 2004. This species usually breeds in woodland pools and has not been previously reported to breed in tires. This collection is presumed to be incidental and does not suggest that *Ps. columbiae* was regularly present in tires during the survey period.

There were no collections of *Oc. japonicus* throughout this survey, nor had this species been reported in Alabama prior to this survey. The previously reported southernmost collection of this species was in Fulton County, Georgia (Gray et al. 2005). However, in the summer of 2005 a single adult *Oc. japonicus* female turned up in a CDC gravid trap in Jackson County, AL (Mullen 2005). *Aedes albopictus* was the only mosquito collected in the larval stage from tire sites in Jackson County during the tirebreeding mosquito survey in Alabama in 2004.

Aedes albopictus was collected at every tire site sampled in this survey. Apparently this species has been successful at establishing itself throughout Alabama. Since *Ae. aegypti* was not collected at all in this survey, the implication is that *Ae. albopictus* has *Ae. aegypti* as the dominant tire-breeder in Alabama. This has similarly been observed by others, most notably in some habitats in Florida (O'Meara et al. 1992), South Carolina (Richardson et al. 1995), and Louisiana (Nasci 1995).

Ochlerotatus triseriatus overlapped with *Ae. albopictus* in 39 of the 67 counties sampled. Previous studies have suggested that *Ae. albopictus* would displace *Oc.*

triseriatus because of its competitive advantage in both larval development time and its ability to hatch in high densities (Ho et al. 1989, Edgerly et al. 1993). However, Moore (1999) stated that temporal and spatial differences between these two species would decrease the likelihood of *Oc. triseriatus* being displaced by *Ae. albopictus*. The data in this survey showed that many natural container-breeders like *Oc. triseriatus* and *Or. signifera* readily oviposited in tires at the same sites in which large collections of *Ae. albopictus* were collected. This may be attributed to the fact that urbanization has reduced natural breeding sites, directly affecting breeding habits of certain tree-hole breeding mosquitoes.

Since *Ae. albopictus* is still considered a potential vector in West Nile Virus transmission and is considered the number one nuisance mosquito in many urban areas of the southeastern United States, its distribution is relevant in assessing the potential public health risks. Because the public is aware of West Nile Virus, better strategies need to be implemented to control the "tire problem". This is already apparent in some of the counties in Alabama that have their own vector control units or mosquito control programs. In the cities of these counties, there is rapid turnover of tires. The tires are removed in a timely fashion so that they are not producing large populations of mosquitoes. Thus, educating the public on the importance that tires play in the production of mosquitoes can influence action to clean up tire sites.

This study provides a baseline for other invasive mosquitoes that might become established in Alabama. Even though *Ae. aegypti* once considered to be the dominant tire-breeding mosquito in Alabama, we have no reported baseline for its distribution in tires in Alabama. With the recent detection of *Oc. japonicus* in Alabama, there is the possibility of this introduced species becoming established in the state. Since *Oc. japonicus* readily develops in tires, the geographic spread of this species and its possible impact on Alabama's tire-breeding mosquito fauna can be monitored in the future based on the baseline information provided.

Literature Cited

- Alto BW, Julian SA. 2001. Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae): implication for range expansion. *J Med Entomol* 38:646-656.
- Andreadis TG. 1988. A survey of mosquitoes breeding in used tire stockpiles in Connecticut. J Am Mosq Control Assoc 4:256-260.
- Baumgartner DL. 1988. Suburban accumulations of discarded tires in northeastern Illinois and their associated mosquitoes. *J Am Mosq Control Assoc* 4:500-508.
- Beier JC, Travis M, Patricoski C, and Kranzfelder J. 1983. Habitat segregation among larval mosquitoes (Diptera: Culicidae) in tire yards in Indiana, USA. *J Med Entomol* 20:76-80.
- Centers for Disease Control and Prevention. 1987. *Aedes albopictus* infestation—United States. *MMWR* 36:769-773.
- Craig, GB Jr. 1980. *Aedes atropalpus* introduced into Indiana: Is this mosquito a potential vector of arboviruses? *Proc Ind Vector Control Assoc* 4:3-7.
- Darsie RF Jr., Ward RA. 1981. *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*. Salt Lake City, UT: American Mosquito Control Association.
- Darsie RF Jr., Ward RA. 2005. *Identification and Geographical Distribution of the Mosquitoes of North America, North of_Mexico*. Gainesville, FL: American Mosquito Control Association.
- Eads RB. 1976. An unusual larval habitat for Culiseta melanura. Mosq News 36:544.
- Edgerly JS, Willey MS, Livdahl TP. 1993. The community ecology of *Aedes* egg hatching: implications for a mosquito invasion. *Ecol Entomol* 18:123-128.

- Edgerly JS, Willey MS, Livdahl TP. 1999. Intraguild predation among larval treehole mosquitoes, *Aedes albopictus*, *Ae. aegypti*, and *Ae. triseriatus* (Diptera: Culicidae), in laboratory microcosms. *J Med Entomol* 36:394-399.
- Gray EW, Harrison BA, Womack ML, Kerce J, Neely CJ, Noblet R. 2005. Ochlerotatus japonicus japonicus (Theobald) in Georgia and North Carolina. J Am Mosq Control Assoc 21:144-146.
- Haramis LD. 1984. *Aedes triseriatus*: a comparison of density in tree holes vs. discarded tires. *Mosq News* 44:485-489.
- Haverfield LE, Hoffman BI. 1966. Used tires as a means of dispersal of *Aedes aegypti* in Texas. *Mosq News* 26:433-435.
- Ho BC, Ewert A, Chew L. 1989. Interspecific competition among Aedes aegypti, Ae. albopictus, and Ae. triseriatus (Diptera: Culicidae): larval development in mixed cultures. J Med Entomol 26:615-623.
- Jamieson, DH, Olson LA, Wilhide JD. 1994. A larval mosquito survey in northeastern Arkansas including a new record for *Aedes albopictus*. J Am Mosq Control Assoc 10:236-239.
- Juliano SA. 1988. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? *Ecology* 79:255-268.
- Joy JE, Hildreth-Whitehair A. 2000. Larval habitat characterization for Aedes triseriatus (Say), the mosquito vector of La Crosse encephalitis in West Virginia. *Wild Environ Med* 11:79-83.
- Joy JE, Hanna AA, Kennedy BA. 2003. Spatial and temporal variation in the mosquitoes (Diptera: Culicidae) inhabiting waste tires in Nicholas County, West Virginia. J Med Entomol 40:73-77.
- Lampman RS, Hanson S, Novak R. 1997. Seasonal abundance and distribution of mosquitoes at a rural waste tire site in Illinois. J Am Mosq Control Assoc 13:193-200.
- Moore CG. 1999. *Aedes albopictus* in the United States: current status and prospects for further spread. *J Am Mosq Control Assoc* 15:221-227.
- Morris CD, Robinson JW. 1994. Distribution of mosquito larvae in waste tire pile in Florida--an initial study. *J Am Mosq Control Assoc* 10:339-343.
- Mount RH. 1975. *The Reptiles and Amphibians of Alabama*. Auburn, AL. Auburn Printing Company.
- Mullen GR. 2005. First report of Ochlerotatus japonicus in Alabama. Alabama Vector Management Society Newsletter. 15 (2):2.
- Novak RJ, Steinly BA, Webb DW, Haramis L, Clarke J, Farmer B, and Cieslik R. 1990 Penetration rate of two pesticide carriers at a large used-tire storage facility in Chicago, Illinois. *J Am Mosq Control Assoc* 13:193-200.
- O'Meara, GF and Karsnick GJ. 1970. Dietary and genetic control of the expression of autogenous reproduction in *Aedes atropalpus* (Coq.) (Diptera: Culicidae). *J Med Entomol* 7:328-324.
- O'Meara GF, Evans LF, Gettman AD, and Cuda JP. 1995. Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. *J Med Entomol* 32:554-562.
- O'Meara GF, Gettman AD, Evans LF, Jr, Curtis GA. 1993. The spread of *Aedes* albopictus in Florida. American Entomologist 39:163-172.
- O'Meara GF, Gettman AD, Evans LF, Scheel FD. 1992. Invasion of cemeteries in Florida by *Aedes albopictus*. J Am Mosq Control Assoc 8: 1-10.

- Pratt JJ JR, Heterick RH, Harrison JB, Haber L. 1946. Tires as a factor in the transportation of mosquitoes by ships. *Mil Surgeon* 99:785-788.
- Reiter P, Sprenger D. 1987. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. *J Am Mosq Control Assoc* 3:494-501.
- Restifo RA, Lanzaro GC. 1980. The occurrence of *Aedes atropalpus* (Coquillett) breeding in tires in Ohio and Indiana. *Mosq News* 40:292-294.
- Sprenger D, Wuithiranyagool T. 1986. The discovery and distribution of *Aedes* albopictus in Harris County, Texas. J Am Mosq Control Assoc 2: 217-218.
- Wilmot TR, Zeller DS, Merritt RW. 1992. A key to container-breeding mosquitoes of Michigan (Diptera: Culicidae), with notes on their biology. *The Great Lakes Entomol* 25:137-148.

Mosquito								
Species	May	June	July	Aug.	Sept.	Oct.	Totals	%
Aedes albopictus	129	1832	3234	2088	1451	429	9163	71
Culex territans	29	135	171	296	220	192	1043	7.5
Ochlerotatus triseriatus	0	190	370	195	148	18	921	7.1
Culex restuans	44	393	94	156	62	28	777	5.2
Culex salinarius	15	58	121	61	85	13	353	3.5
Orthopodomyia signifera	0	51	132	32	73	28	316	2.7
Culex quinquefasciatus	3	9	27	8	93	36	176	1.2
Ochlerotatus atropalpus	0	35	39	30	29	0	133	0.9
Toxorhynchites rutilus	0	14	28	22	12	7	83	0.7
Anopheles spp.	1	3	10	8	0	1	23	0.2
Psorophora columbiae	0	13	8	0	0	0	21	0.2
Anopheles punctipennis	0	10	0	0	0	0	10	< 0.1
Anopheles quadrimaculatus	0	3	0	0	0	0	3	< 0.1
Totals	221	2746	4234	2896	2173	752	13022	100

Table 1. Total numbers and percentages of mosquito species collected from tires in Alabama by month, based on the 2004-2005 larval survey. Species are listed in descending order of the total numbers of each species collected.

					Month		
County	Mosquito Species	May	June	July	August	September	Octobe
Autauga	Aedes albopictus		58	160	31	52	
	Culex territans		1				
	Ochlerotatus triseriatus			12		20	
	Culex restuans		13		4		
	Culex salinarius					4	
	Orthopodomyia signifera			11		9	
	Culex quinquefasciatus			5			
	Toxorhynchites rutilus			7	2		
	Anopheles quadrimaculatus		2				
	Totals	0	74	190	37	82	0
Baldwin	Aedes albopictus		9	33	14	1	1
	Culex territans			1	4		
	Ochlerotatus triseriatus		1	Э	7		
	Culex restuans						
	Culex salinarius		1	13			
	Orthopodomyia signifera			5		5	
	Totals	0	11	55	20	9	1
Barhour	Andres alhomictus		50	117	000		72
	Culex territans)	5			1
	Ochlerotatus triseriatus			2	Ś		

Table 2. Total numbers of mosquito species collected by county and month from tires in Alabama, based on the 2004-2005 larval survey. The sixty-seven Alabama counties are listed in alphabetical order.

32	e	e	•	5 1 11
0	•	0	0	62 62
3 217	•	e	0	68 5 83
5 5 13 4	49 38	0	0	38 5 11 56
20 70	20 6 1 7 43	20 11 8 1	09 09	5 6 1
•	•	o	0	•
Culex restuans Culex salinarius Orthopodomyia signifera Totals	Aedes albopictus Culex territans Ochlerotatus triseriatus Ochlerotatus atropalpus Totals	Aedes albopictus Ochlerotatus triseriatus Culex restuans Anopheles quadrimaculatus Totals	Aedes albopictus Totals	Aedes albopictus Culex territans Culex restuans Culex salinarius Culex quinquefasciatus Totals
	Bibb	Blount	Bullock	Butler

Culex sa Culex sa Totals Chambers <u>Aedes al</u> Culex ter Ochlerot	linarius		-				
Totals Chambers <u>Aedes ali</u> Culex ten Ochlerot				11		L	
Chambers <u>Aedes ali</u> Culex ten Ochlerot		0	58	58	33	85	30
Culex ter Ochlerot	bopictus			26	18		
Ochlerot	rritans				5		
	tatus triseriatus				8		
Totals		0	0	26	31	0	0
Chilton <u>Aedes al</u>	bopictus		11	116	29	26	7
Culex ter	rritans						11
Ochlerot	tatus triseriatus		10		4	6	
Culex re	stuans				7		
Culex sa	linarius						5
Culex qu	<i>iinquefasciatus</i>					2	
Totals		0	21	116	40	37	23
Cherokee <u>Aedes al</u>	bopictus		17				
Culex ter	rritans		15				
Culex re	stuans		21				
Ochlerot	tatus atropalpus		ς				
Anopheli	es punctipennis		б				
Anopheli	es quadrimaculatus		7				
Totals		0	61	0	0	0	0

Choctaw	Aedes albopictus Totals	0	10 10	•	0	0	0
Clarke	Aedes albopictus Culex territans Ochlerotatus triseriatus Ochlex restuans Orthopodomyia signifera Toxorhynchites rutilus Anopheles spp. Totals	•	4 m Γ	38 4 17 5 105	40	•	•
Clay	Aedes albopictus Totals	0	0	15 15	0	0	0
Cleburne	Aedes albopictus Culex territans Ochlerotatus triseriatus Totals	•	5 12	91 15 106	56 3 59	38 2 1 41	•
Coffee	Aedes albopictus Culex territans Ochlerotatus triseriatus Ulex restuans Orthopodomyia signifera Culex quinquefasciatus Totals	•	36 13 49	123 3 4 137	85 85	121 1 122	62 1 23

Iotals 0 42 0 20 01 3 ecul $\frac{4ees albopictus}{0 chlerotauts triseriatus}$ $\frac{41}{5}$ $\frac{18}{5}$ $\frac{13}{2}$ $\frac{13}{8}$ Sa $\frac{7}{0 chlerotauts triseriatus}$ $\frac{4}{5}$ $\frac{1}{3}$ $\frac{13}{8}$ $\frac{1}{3}$ $\frac{1}{$	olbert	Aedes albopictus Culex territans Ochlerotatus triseriatus Culex salinarius Orthopodomyia signifera	c	42		5 56	7 2 2 2	0 m u
		l otals	•	42	•	26	9	n
TotalsTotals004621210sa $\overline{Aedes albopictus}$ $\overline{43}$ $\overline{43}$ $\overline{10}$ $\overline{0}$ $\overline{0}$ $\overline{0}$ ington $\overline{Aedes albopictus}$ $\overline{54}$ 129 131 $\overline{3}$ $\overline{36}$ ington $\overline{Aedes albopictus}$ $\overline{54}$ 129 131 $\overline{3}$ $\overline{36}$ $\overline{Culex territons}$ $\overline{3}$ $\overline{2}$ 1 1 1 1 $\overline{Culex territons}$ 2 129 131 3 $\overline{6}$ $\overline{Culex territons}$ 2 19 6 6 $\overline{Culex salinarius}$ 2 131 151 3 57 $\overline{Culex salinarius}$ 0 61 131 151 3 57 $\overline{Culex salinarius}$ 2 2 2 2 2 $\overline{Culex salinarius}$ 2 2 2 36 $\overline{Culex salinarius}$ 2 2 2 36 $\overline{Culex salinarius}$ 2 2 2 2 $\overline{Culex salinarius}$ 2 2 2 3 $\overline{Culex salinarius}$ 2 2 2 3 $\overline{Culex salinarius}22236\overline{Culex salinarius}2223\overline{Culex salinarius}$	necuh	Aedes albopictus Ochlerotatus triseriatus			41 5	18 3	13 8	
sa $4edes albopictus$ 43 0 0 0 0 0 $Totals$ 0 0 43 0 0 0 0 ington $Aedes albopictus$ 54 129 131 3 36 ington $Aedes albopictus$ 2 11 3 36 $Culex territans2111111Culex territans21966Culex restants21966Culex restants21966Culex restants21966Culex restants21966Culex restants21966Culex restants21966Culex restants219357Culex restants0600661013115135710 mbav4edes albopictus000000$		Totals	•	0	46	21	21	0
Totals00430000ington $Aedes albopictus$ 54 129 131 3 36 $Culex territans32111Culex territans21111Culex territans21141Culex territans21111Culex restants21966Culex restants21966Culex restants21966Culex restants21966Culex restants21966Culex salinarius21966Culex salinarius21966Culex salinarius219357Culex salinarius131151357Culex salinarius131151357Culex salinarius00000$	sa	Aedes albopictus			43			
$ \begin{array}{llllllllllllllllllllllllllllllllllll$		Totals	0	0	43	0	0	0
$\begin{tabular}{cccccccccccccccccccccccccccccccccccc$	ington	Aedes albopictus		54	129	131	3	36
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		Culex territans		ŝ				
$\begin{tabular}{cccccccccccccccccccccccccccccccccccc$		Ochlerotatus triseriatus		7				11
Culex salinarius 2 19 6 Orthopodomyia signifera 2 4 4 Orthopodomyia signifera 0 61 131 151 3 57 shaw Aedes albopictus 0 0 0 82 0 0		Culex restuans				1		
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		Culex salinarius		2		19		9
Totals 0 61 131 151 3 57 shaw Aedes albopictus 82 9 9 9 9 Totals 0 0 0 82 0 0 0		Orthopodomyia signifera			2			4
nshaw Aedes albopictus 82 Totals 0 0 0 82 0		Totals	0	61	131	151	3	57
Totals 0 0 0 82 0 0	nshaw	Aedes albopictus				82		
		Totals	0	0	0	82	0	0

	7		5			٢	21	13			5				39					0		0	
47		2		8	39	96	144	1	32		20	2	7		206					0		0	
31	5					36	135		19		14		6		177					0	48	48	
71		12				83	179		35	7	14		1		236					0	34	34	83
27		12	28			67	109	44	28	8		1	1		191	22	-	ю		26		0	98
						0	80		5					1	86					0		0	
Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex restuans	Culex salinarius	Culex quinquefasciatus	Totals	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex salinarius	Orthopodomyia signifera	Culex quinquefasciatus	Toxorhynchites rutilus	Anopheles punctipennis	Totals	Aedes albopictus	Culex restuans	Culex quinquefasciatus	Ochlerotatus atropalpus	Totals	Aedes albopictus	Totals	Aedes albopictus
Cullman							Dale									Dallas					Dekalb		Elmore

	Totals	0	98	130	0	0	0
Escambia	Aedes albopictus		22	60	47	46	41
	Culex territans			15			14
	Ochlerotatus triseriatus					4	
	Culex restuans		8				
	Culex salinarius		10		2	1	
	Totals	0	40	75	49	51	55
Etowah	Aedes albopictus		3	50		2	16
	Culex territans						14
	Ochlerotatus triseriatus			13			
	Culex restuans		7		42	58	
	Culex salinarius					4	
	Ochlerotatus atropalpus		12	32	30	29	
	Toxorhynchites rutilus			2			
	Anopheles punctipennis						1
	Totals	•	17	76	72	93	31
Fayette	Aedes albopictus				15		
	Totals	0	0	0	15	0	0
Franklin	Aedes albopictus	2	2	5			
	Totals	2	2	5	0	0	0
Geneva	Aedes albopictus		30	16			
	Ochlerotatus triseriatus		26	59			
	Culex salinarius		e	5			
	Totals	0	59	80	0	0	0

C	1 - 1		0	Ę	r		
Ureene	Aeaes albopictus		69	4/	/	3 3	
	Culex territans					1	
	Culex salinarius			38		58	
	Culex quinquefasciatus					2	
	Toxorhynchites rutilus		1				
	Anopheles punctipennis			З	1		
	Totals	0	70	88	æ	94	0
Hale	Aedes albopictus		55	106			
	Culex territans				7		
	Culex salinarius				Э		
	Totals	0	55	106	S	0	0
Понти	Andres alkonintus		52	11	102	12	L C
TICHTY	manual anophicing		C C	Ŧ	C01	f	11
	Culex territans				18		
	Ochlerotatus triseriatus			Э			
	Culex restuans		31				
	Culex salinarius				1	7	
	Culex quinquefasciatus						8
	Toxorhynchites rutilus		1	2	1		
	Anopheles quadrimaculatus		1				
	Totals	0	86	46	123	45	35
Houston	Aedes albopictus	38					
	Culex territans	20					
	Culex restuans	24					
	Culex quinquefasciatus	б					
	Anopheles punctipennis	1					

	Totals	86	0	0	0	0	0
Jackson	Aedes albopictus Totals	0	55 55	0	0	0	0
Jefferson	Aedes albopictus Culex territans Toxorhynchites rutilus	c	P 0 0	c	c	4	
Lamar	1 otals <i>Aedes albopictus</i> <i>Culex territans</i> <i>Culex restuans</i> <i>Anopheles punctipennis</i>	-	11 5 1 1	-	-	•	-
	Totals	0	41	0	0	0	0
Lauderdale	Aedes albopictus Culex territans Ochlerotatus triseriatus	ω4	14	13 15 25	93	85	
	Culex restuans Toxorhynchites rutilus Anopheles quadrimaculatus	0 4	-			_	
	Totals	13	15	55	93	86	0
Lawrence	Aedes albopictus Culex restuans Culex salinarius		46 5 2				
	Anopnetes punctipennts Totals	0	с 69	0	0	0	0

Lee	Aedes albopictus		42	109	62	49	23
	Culex territans				5	7	13
	Ochlerotatus triseriatus		10	12			
	Culex restuans				23		
	Orthopodomyia signifera		1				4
	Toxorhynchites rutilus		1				1
	Totals	0	54	121	107	51	41
Limestone	Aedes albopictus		24				
	Culex territans		5				
	Ochlerotatus triseriatus		С				
	Culex restuans		22				
	Anopheles punctipennis		1				
	Totals	0	55	0	0	0	0
Lowndes	Aedes albopictus				3		
	Culex territans				5		
	Culex quinquefasciatus				1		
	Totals	0	0	0	6	0	0
Macon	Aedes albopictus				38	48	
	Culex territans					2	
	Ochlerotatus triseriatus				2	20	
	Culex salinarius				Э		
	Orthopodomyia signifera					14	
	Toxorhynchites rutilus				2		9

	Totals	0	0	0	45	84	9	
Madison	Aedes albopictus		31					i i
	Culex restuans		5					
	Culex salinarius		-					
	Culex quinquefasciatus		б					
	Anopheles punctipennis		Э					
	Totals	0	43	0	0	0	0	
Marengo	Aedes albopictus			50				1
	Culex territans			43				
	Ochlerotatus triseriatus			5				
	Totals	0	0	98	0	0	0	
Marion	Aedes albopictus	9	17	172		29	20	I
	Culex territans					4	16	
	Ochlerotatus triseriatus		8	39		ŝ		
	Culex restuans		5			З		
	Culex quinquefasciatus		1					
	Toxorhynchites rutilus		1					
	Totals	9	32	211	0	39	36	
Marshall	Aedes albopictus	2	12	54	8	25		I
	Culex territans		7			ŝ		
	Ochlerotatus triseriatus			8		7		
	Culex restuans	1	22	1		1		
	Culex salinarius			19		13		

	0	63	10	7	8		1	23		112						0	2			5	
ς N	57	99	1	11				5	ю	86	33	4	1		19	57	94	44	16		
	×	37	14	7				7	7	62	66	27			7	100	66	12	46		6
Ś	87	45	11			4		11	10	81	82			10		92	107	22	39		
L	43	46	-	18	6	9	8	ε	7	93						0	144	13	1		1
	ε									0						0					
Orthopodomyia signifera Culex quinquefasciatus Toxorhynchites rutilus	Totals	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex restuans	Culex salinarius	Orthopodomyia signifera	Culex quinquefasciatus	Toxorhynchites rutilus	Totals	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex salinarius	Orthopodomyia signifera	Totals	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex restuans	Culex salinarius
		Mobile									Monroe						Montgomery				

4	11					0				0	21	75										96
	154					0				0	46	25	5					7				78
ξ	136					0				0	120	91	5	47	21					4		288
21 3	199					0	38	22	7	67	247	12	17	12			20		8	7		318
1	160	т »	16	9	7	40				0	88	15	1	24		1			13			142
	0					0				0												0
Orthopodomyta signifera Ochlerotatus atropalpus Toxorhynchites rutilus	Totals	Aedes albopictus Culex territans	Culex restuans	Culex salinarius	Culex quinquefasciatus	Totals	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Totals	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex restuans	Culex salinarius	Orthopodomyia signifera	Culex quinquefasciatus	Toxorhynchites rutilus	Psorophora columbiae	Anopheles punctipennis	Anopheles quadrimaculatus	Totals
		Morgan					Perry	•			Pickens											

● ● 4 4 ∞ ∞	3 3 3 3 1 9 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0 1 1 1 1 1 1 1 1 1 1	30 30 30 17 17 17 86 86	18 18 18 18 19 19 19 19 19 19 19 19	16 16 16 16 16 16 16 16	• • • •	edes albopictus otals edes albopictus ulex territans ulex restuans otals otals corhynchites rutilus otals otals clevatus triseriatus chlerotatus triseriatus chlerotatus triseriatus otals
	38 3	37	38			es albopictus hopodomyia signifera
	04	72	30	c	c	upouvinytu signijeru Je
	J					" in his and in another
	ŝ					opodomyia signifera
	,					
	38	37	38			albopictus
	0	ļ	(
)))	>	1	,	3	
8	36	86	32	0	0	
	ŋ	T	71			ouomynu siznijeru
	"	-	c1			odomnia sionifera
			4			alinarius
		2)			
		43	5			otatus triseriatus
	7					erritans
	(
8	31	42	11			lbopictus
4	20	17	48	10	0	
	1					nchites rutilus
4	IY	1/	40	٧		popicius
4	19	17	48	0		honictus
0	•	0	•	35	0	
				ŝ		tuans
						ritans
				-		
				31		bopictus
0	0	30	18	16	0	
		30	18	16		bopictus

		0							0				0	22					L			29		
		0							0				0	131		5					1	137		
		0							0				0	57			2					59		
		0							0	102	20	7	124	52		8	10					70		
1		53	62		20			1	83				0	51	1	0	34	8		1		76	Ŷ	، د
	6	6		5		17	15		37				0									0		
Ochlerotatus triseriatus	Culex restuans	Totals	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex restuans	Culex salinarius	Toxorhynchites rutilus	Totals	Aedes albopictus	Culex territans	Toxorhynchites rutilus	Totals	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex restuans	Culex salinarius	Orthopodomyia signifera	Culex quinquefasciatus	Toxorhynchites rutilus	Totals	Aedes alhonictus	Culex restrans
			Talladega							Tallapoosa				Tuscaloosa									Walker	

•	•	e	0
e	•	e	0
•	51 8 59	•	•
0	57 57	43 43	•
29 8 7	60 1 61	•	11 15 49
e	e	•	0
Orthopodomyia signifera Totals	Aedes albopictus Ochlerotatus triseriatus Culex salinarius Totals	Aedes albopictus Totals	Aedes albopictus Culex restuans Culex salinarius Totals
	Washington	Wilcox	Winston

-2005	
2004-	
n the	
sed or	
r, bas	
ctobe	
gh O	cted.
throu	colle
May	ecies
from	ch sp(
vals	ofea
c inter	lbers
-week	l nur
by 2-	e tota
itoes	of th
nbsou	order
ling n	ding .
breed	escen
f tire-	l in de
ice of	listed
absei	s are
ce or	pecie
resen	ey. S
З. Р	Surv
Table	larval

					Σ	onth					
Mosquito Species	May	Ju	ne	Ju	ly	AL	lg.	Sej	ot.	Õ	ct.
Aedes albopictus	+	+	+	+	+	+	+	+	+	+	+
Culex territans	+	+	+	+	+	+	+	+	+	+	
Ochlerotatus triseriatus		+	+	+	+	+	+	+	+	+	
Culex restuans	+	+	+	+	+	+	+	+		+	
Culex salinarius	+	+	+	+		+		+		+	
Orthopodomyia signifera			+	+	+	+		+	+	+	
Culex quinquefasciatus	+	+			+	+	+	+		+	
Ochlerotatus atropalpus		+			+	+	+	+			
Toxorhynchites rutilus			+	+		+	+	+		+	
Anopheles spp.	+	+	+		+					+	
Psorophora columbiae		+	+	+							
Anopheles punctipennis	+	+									
Anopheles auadrimaculatus		+									

		Physiogra	phic Regions of Alabam	la	
Mosquito Species	East Gulf Coastal Plains	Alabama Valley and Ridge	Cumberland Plateau	Highland Rim	Piedmont Upland
Aedes albopictus	+	+	+	+	+
Culex territans	+	+	+	+	+
Ochlerotatus triseriatus	+	+	+	+	+
Culex restuans	+	+	+	+	+
Culex salinarius	+	+	+	+	
Orthopodomyia signifera	+	+	+	+	
Culex quinquefasciatus	+	+	+	+	
Ochlerotatus atropalpus	+	+			
Toxorhynchites rutilus	+	+	+	+	+
Psorophora columbiae	+				
Anopheles punctipennis	+	+		+	
Anopheles quadrimaculatus		+			

by 2-week intervals from May through	
phic region	
y physiogra	
s collected b	
mosquitoes	y.
re-breeding	larval surve
absence of ti	2004-2005
Presence or	based on the
Table 5.	October,

						M	onth					
Physiogeographic Region	Mosquito Species	May	Jur	le	Jul	[y	At	<u>6</u>	Sel	pt.	0	t.
East Gulf Costal Plain	Aedes albopictus	+	+	+	+	+	+	+	+	+	+	+
	Culex territans	+	+	+	+	+	+	+	+	+	+	+
	Ochlerotatus triseriatus		+	+	+	+	+	+	+	+	+	
	Culex restuans	+	+	+	+	+	+	+		+	+	
	Culex salinarius		+	+	+	+	+	+	+	+	+	
	Orthopodomyia signifera			+	+	+	+	+	+	+	+	
	Culex quinquefasciatus	+	+			+	+		+		+	
	Ochlerotatus atropalpus			+	+							
	Toxorhynchites rutilus			+	+	+	+	+	+		+	
	Psorophora columbiae			+	+							
	Anopheles punctipennis	+	+									
	Anopheles spp	+	+		+		+					
Alabama Valley and Ridge	Aedes albopictus		+	+	+	+	+	+	+	+	+	+
	Culex territans	+	+							+	+	+
	Ochlerotatus triseriatus		+	+	+	+	+					
	Culex restuans	+	+				+	+	+			
	Culex salinarius	+			+				+			
	Orthopodomyia signifera				+				+			
	Ochlerotatus atropalpus		+		+	+	+	+	+			
	Toxorhynchites rutilus		+		+							
	Anopheles punctipennis		+									
	Anopheles quadrimaculatus		+									
	Anopheles spp											+
Cumberland Plateau	Aedes albopictus	+	+	+	+	+	+	+	+	+	+	
	Culex territans			+			+		+			

						+			+									
		+		+		+								+				
+	+			+	+	+	+	+				+		+	+	+		
						+	+							+				
	+					+	+							+	+	+		
_	_	_	_	_	_	+	+	_	_	_	_	_		+	_	+	_	_
+	+	+	+			+	+	+						+	+	+		+
+	+		+			+	+	+				+		+	+		+	
+	+	+				+	+		+	+	+	+	+	+				
	+					+	+		+				+					
Ochlerotatus triseriatus	Culex restuans	Culex salinarius	Orthopodomyia signifera	Culex quinquefasciatus	Ochlerotatus atropalpus	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex restuans	Culex salinarius	Culex quinquefasciatus	Toxorhynchites rutilus	Anopheles punctipennis	Aedes albopictus	Culex territans	Ochlerotatus triseriatus	Culex restuans	Toxorhynchites rutilus
						ghland Rim								edmont Upland				



Figure 1. Map of Alabama Counties reproduced from the Department of Geography, College of Arts and Sciences, the University of Alabama.



Figure 2. Map of Alabama physiographic regions reproduced from the Department of Geography, College of Arts and Sciences, the University of Alabama.



Figure 3. Alabama counties in which larvae were collected from tires, shown in gray. a, 2004. b, 2005.



Figure 4. Map of Alabama showing the locations of tire sites sampled in each county during the 2004-2005 larval survey. A total of 169 tire sites was sampled.



Figure 5. Distribution, by county, of tire-breeding mosquitoes in Alabama, based on 2004-2005 larval survey. *Aedes albopictus, Cx. quinquefasciatus, Cx. restuans*, and *Cx.*

salinarius.



Figure 6. Distribution, by county, of tire-breeding mosquitoes in Alabama, based on 2004-2005 larval survey. *Culex territans, Oc. atropalpus, Oc. triseriatus, and Or. signifera.*



Psorophora columbiae



Anopheles punctipennis



Toxorhynchites rutilus



Anopheles quadrimaculatus

Figure 7. Distribution, by county, of tire-breeding mosquitoes in Alabama, based on 2004-2005 larval survey. *Psorophora columbiae*, *Tx. rutilus, An. punctipennis*, and *An. quadrimaculatus*.



Anopheles spp.

Figure 8. Distribution, by county, of tire-breeding mosquitoes in Alabama, based on 2004-2005 larval survey. *Anopheles* spp.



Figure 9. Occurrence of tire-breeding mosquitoes by 2-week intervals from May through October, based on the 2004-2005 larval survey.



Figure 10. Map of Alabama showing the 5 physiographic regions and the number of tire sites sampled per county, based on the 2004-2005 larval survey.

EVALUATION OF THE MOSQUITO ATTRACTANT OCTENOL FOR ENHANCING COLLECTIONS OF TIRE-BREEDING MOSQUITOES IN PROPANE-POWERED TRAPS

Objectives

- To determine the species of mosquitoes that are attracted to octenol in an urban setting.
- To determine if propane-powered traps baited with octenol are an effective tool in monitoring tire-breeding mosquito populations.
- To determine if propane-powered traps are effective in reducing populations of urban mosquitoes below nuisance levels.

1-octeno-3-ol, (octenol) is a volatile compound that has been isolated from many natural sources, including both invertebrates and vertebrates. Octenol is an 8-carbon mono-unsaturated alcohol with two isomers. Hall et al. (1984) was the first to isolate octenol from oxen breath, and the substance was first successfully used as an attractant in the tsetse control programs in Zimbabwe and other parts of Africa (Torr 1994). Ceratopogonids (Kline 1994), tabanids (French and Kline 1989), oestrids (Anderson 1989), and mosquitoes (Takken and Kline 1989; Kline et. al 1990, 1991) have been shown to be similarly attracted to traps baited with octenol. Field studies conducted in a variety of ecological habitats to evaluate the attractiveness of octenol to mosquitoes, including estuarine ecosystems (Kline et al. 1990, 1991; Takken and Kline 1989, Rueda et al. 2001), freshwater swamps (Takken and Kline 1989), phosphate-mined areas (Kline et al. 1990), and irrigated ricelands (Kline et al. 1991), have shown that many mosquito species are attracted to octenol. Although Kline (1994) found that *Ochlerotatus sollicitans, Oc. taeniorhynchus, Oc. triseriatus, Culex salinarius,* and *Mansonia titillans* have shown a positive response to octenol-supplemented traps, other studies showed no effects on collections when octenol alone was used as an attractant (Kline 1994, Kline 2002). In fact, octenol reportedly can cause a negative response in *Culex* (Kline 1994, Mboera et al. 2000, Burkett et al. 2001). Other studies have shown that the combination of carbon dioxide and octenol results in a synergistic effect in the response of many mosquito species (Kemme et al. 1993, Kline 1994, Kline and Mann 1998).

In recent years trapping devices have been developed utilizing catalytic combustion of propane to produce CO₂, heat, and water vapor as a means of managing some mosquito populations (Kline 2002). The Mosquito Magnet Pro^{TM} (MMP) is one such propane-powered trap that uses a counterflow technologyTM to emit a plume of CO₂, heat, and water vapor in combination with octenol. Studies have shown that these octenol-supplemented traps often collect large numbers and a diversity of mosquito species when operated in proximity to salt marshes (Takken and Kline 1989, Kemme et al. 1993, and Rueda et al. 2001). Other studies have shown no differences in collections when octenol is used in other ecological habitats (Burkett et al. 2001, Rueda et al. 2001 and Shone et al 2003). Rueda et al. (2001) found that when trapping in a salt marsh in North Carolina

with light traps baited with CO_2 , light and octenol, collections of important vectors of Eastern Equine Encephalitis virus were increased. However, when collecting in a creek flood plain in North Carolina, these authors found no statistically significant difference in mosquito collections with the addition of octenol (Rueda et al. 2001).

The MMP traps, produced by the American Biophysics Corporation, are commercially available to homeowners. The pamphlet that comes with the traps cautions "that recent studies show that Octenol may actually repel the Asian Tiger Mosquito" Aedes albopictus, the number-one nuisance mosquito in many urban communities in the southeastern United States. The source of this statement was unpublished proprietary studies. Only a few studies have addressed the response of Ae. albopictus to octenolsupplemented traps (Shone et al. 2003 and Dennett et al. 2004). Shone (2003) used the Fay-Prince trap baited with CO_2 and CO_2 + octenol to evaluate the ability of the trap to collect Ae. albopictus. They found no statistically significant differences in the response of Ae. albopictus to these two combinations of attractants. Traps using either carbon dioxide alone or CO_2 + octenol were, on the other hand, statistically more attractive to *Ae. albopictus* than were traps that were either unbaited or used only octenol. These results suggested that CO₂ is driving the response of *Ae. albopictus*, not octenol. Shone et al. (2003) did not indicate that octenol was acting as a repellent to *Ae. albopictus*. Thus, there are no published reports that provide a basis for American Biophysics Cooperation's statement.

Propane traps in combination with the attractant octenol have not been extensively evaluated in urban areas. This leaves unanswered questions as to whether or not octenol is attractive or repellent to certain urban mosquitoes. This study was designed to evaluate
the effectiveness of the MMP with and without octenol in attracting mosquitoes typically found in urban environments.

Three field trials were conducted in the communities of Auburn and Phenix City, Alabama, to evaluate octenol. The specific objectives of the study were to determine: (1) the species of mosquitoes attracted to octenol in an urban setting, (2) if the MMP using octenol as an attractant is an effective tool for monitoring mosquito populations, and (3) if the MMP is an effective trap in reducing mosquito populations of urban mosquitoes below nuisance levels.

Study Sites

Field Trial 1

Preliminary field trial 1 was conducted using two MMP traps at an auto-repair shop at Auburn, Alabama. Behind of the auto-repair shop was an outdoor tire-storage area where about 200 discarded tires, ranging from compact-car tires to tractor-trailer tires, were stored. About half of these tires were sheltered in a covered tire rack. Because the tires were protected, the tires did not hold water and subsequently were not sites of mosquito larval development. The remaining tires were stored in an open area adjacent to the tire rack. These tires were either laying flat on the ground or propped up against other tires. Mosquito larvae were observed in many of these tires. A fence covered with kudzu (*Pueraria montana*) bordered the perimeter of the auto shop just behind the area where the tires were located.

Field Trial 2

Preliminary field trial 2 was conducted using two MMP traps placed in the proximity of 4 greenhouses located on the Auburn University Campus. Two MMP traps were placed 2 meters apart in a low-lying drainage area that collected runoff from the irrigation system used in the greenhouses. Small runoff pools were formed in this area allowing mosquito breeding. Loblolly pine (*Pinu taeda*), American holly (*Ilex opaca*), pin oak (*Quercus palustris*), and willow oak (*Quercus* phellos) were the predominant vegetation in the drainage area. Ornamental ponds that held about 4-6 inches of standing water were located near the greenhouses and were possible mosquito breeding sites.

Field Trial 3

The salvage yard is a 3-acre fenced lot with cars, car parts, and discarded tires scattered throughout. The site mostly consisted of wrecked cars that were lined up in rows in the front and back of the lot. At the front end of the lot approximately 25 tires were stacked horizontally on top of each other and held very little rain water. Along the far back fence corner there were approximately 40 tires of varying sizes that consistently held water during the 8-week field trial. Mosquito larvae were observed in these tires. In the center of the salvage yard a wooded area consisting of predominantly sweet gum (*Liquidamtar styraciflua*) and swamp willow (*Salix caroliniana*) separated the front lot from the back lot. Other vegetation in this area included tulip tree (*Liriodendron tulipifera*), goldenrod (*Solidago* spp.), cattails (*Typha latifolia*), sedge (*Carex firma*), and rush (*Juncus patens*). Approximately 500 tires were scattered throughout the adjacent wooded area. These tires were lying on their sides, propped up against one another, or

piled haphazardly on top of one another in tire mounds. Mosquito larvae also were observed in these tires.

Materials and Methods

Field trials were conducted during the summer of 2004 to evaluate the performance of the propane-powered Mosquito Magnet Pro^{TM} trap (Figure 11) (American Biophysics Corporations, East Greenwich, RI). The catalytic combustion of propane, which converts 20 pounds of propane to 60 pounds of CO_2 , generates the power to run the counterflow suction fan for insect entrapment while producing the long-range attractants. Replaceable 1.7-g octenol cartridges (American Biophysics Corporation) were placed in the compartment located at the bottom of the MMP fan unit.

The first two preliminary trials were conducted at two locations in Auburn, AL. Two MMP traps were placed at each trial site. One trap was operated with octenol, whereas the other trap was not. Collection nets were removed and replaced each day. All mosquito collections were brought to the laboratory for identification to species. Each of these two trials was conducted over a 4-week period, the first trial from May 27 to June 23 and the second trial from June 2 to June 23.

The third field trial was conducted at an automobile salvage and tireyard at Phenix City, AL from July 7 to August 16. Four MMP traps were placed 20 meters apart in a 1x2 factorial design in the wooded area located in the center of the salvage yard. Four traps were operated weekly, two traps with octenol and two traps without octenol, throughout the 8-week field trial. Each trap was supplemented with octenol, such that octenol occupied each trapping position at least one time during the field trial. Octenol was replaced at the end of each 7-day period. Mosquito collections were removed at the end of each 7-day period, and the species identified and counted.

Two two-minute landing counts (Figure 12) were taken twice a week between 9:00 and 11:00 am within the salvage yard, with locations randomly selected on each occasion by tossing a stick. The right or left leg from knee down was exposed while a hand-held battery aspirator was used to remove the landing mosquitoes. A two-minute acclimation time was allowed before the landing counts began. The landing counts were averaged by week.

Six tires were randomly sampled each week. All the water was removed from the tires and the mosquito larvae recovered. The samples were brought back to the lab for larval identification. Tires were sampled throughout the trial to determine which mosquito species were breeding in the tires at the salvage yard.

Statistical Analysis

For preliminary field trials 1 and 2 collections with greater than 100 mosquitoes, a repeated measures ANOVA was used to determine if there was a significant difference between the treatments. The repeated measures ANOVA was used because the dependent variable, time, was repeated. For the third field trial collections with greater than 100 mosquitoes treatment (octenol vs. no octenol), position, and week effects were analyzed using a 3-way ANOVA for each species trapped. The 3-way ANOVA was used to determine the effectiveness of octenol in enhancing collections of individual mosquito species. A Tukey's test was used if there were any significant interactions

between the variables tested in the 3-way ANOVA. A Chi-square test was used for species with trap collections less than 100.

A Proc Corr analysis was used to determine if there was any correlation between the mean weekly landing counts for each mosquito species and the collections in the MMP traps.

Results

Preliminary Field Trial 1

A total of 1501 mosquitoes, representing 7 species in 5 genera, was collected during the 4-week field trial (Table 6). A total of 1061 (71%) of the mosquitoes was collected in MMP traps provided with octenol, compared to 440 (29%) collected in the MMP traps without octenol. The most frequently collected species in the octenolsupplemented traps was *Ae. albopictus* (1472/1501), comprising 98% of the collections. Collections of *Ae. albopictus* were 2.5-fold greater with octenol (1051, or 71%) than without octenol (421, or 29%). On 5 individual trap nights, collections of *Ae. albopictus* were significantly enhanced with use of octenol (Figure 13). The next most frequently collected species were *Anopheles punctipennis, Cx. quinquefasciatus, Ae. vexans, Psorophora columbiae*, and *Ochlerotatus triseriatus*.

There was a significant difference between treatments for collections of *Ae. albopictus* [P=.03] as determined by a repeated measures ANOVA (Table 7). There was no significant difference between the treatments of *Cx. quinquefasciatus, Ae. vexans, Ps. columbiae,* and *Oc. triseriatus. Anopheles punctipennis* collections were significantly decreased when octenol was used as the attractant, [P =.01].

Preliminary Field Trial 2

A total of 655 mosquitoes, representing 6 species in 5 genera, was collected during the 3-week field trial (Table 8). A total of 552 (84%) of the mosquitoes was collected using octenol. Only 103 (16%) mosquitoes were collected without octenol. Of these collections 570 (80%) were *Ae. albopictus*, with 489 (86%) collected with octenol, versus 81 (14%) collected without octenol. On 9 individual trap-nights, *Ae. albopictus* collections were significantly enhanced with octenol (Figure 14). The next most frequently collected species were: *An. punctipennis* (35), *Cx. quinquefasciatus* (27) *Ae. vexans* (19), *Oc. triseriatus* (2), and *Ps. columbiae* (2).

There was a significant difference between treatments (octenol vs. no octenol) on the collections of *Ae. albopictus* [P =.02] as determined by a repeated measures ANOVA (Table 9). Octenol significantly increased the collections of *Ae. albopictus* by 6 fold. The treatments were not significant in the collections of *Cx. quinquefasciatus, Oc. triseriatus,* and *Ps. columbiae*. However, the addition of octenol significantly increased the collections of *An. punctipennis* [P=.05] by 3 fold and *Ae. vexans* [P=.05] by 18 fold.

Field Trial 3

A total of 7143 mosquitoes, representing 13 species in 5 genera, was collected over the 8-week study period (Table 10). A total of 5773 mosquitoes (81%) was collected with octenol, versus 1370 (19%) in the MMP traps operated without octenol. Of these collections 5571 (78%) were *Ae. albopictus*, with 4334 (77%) collected with octenol. The next most frequently collected species were, in descending order: *Oc. triseriatus* (1302), Coquillettida perturbans (131), An. punctipennis (35), Ps. columbiae (33), Cx. restuans (29), Cx. salinarius (13), Cx. erraticus (9), Cx. quinquefasciatus (8), An. crucians (5), Ae. vexans (3), Ps. ferox (3), and Cx. territans (1). Culex salinarius and Cx. territans were collected only with MMP traps provided with octenol. All 13 mosquito species trapped, with the exception of *Culex quinquefasciatus*, were collected in greater numbers with octenol than without octenol. There were 4 times more mosquitoes collected with octenol than without octenol.

There were no statistically significant effects between treatment (octenol vs. no octenol) and trap position as detected by the 3-way ANOVA for *Ae. albopictus*, *Oc. triseriatus*, and *Cq. perturbans* (Table 11). However, there were significant effects between treatment and week indicated by the 3-way ANOVA for *Ae. albopictus* and *Oc. triseriatus*. Tukey's test detected a significant difference between week 1 and all other weeks. There were no effects between treatment and week for *Cq. perturbans*.

There was an effect between treatments (octenol vs. no octenol) for collections of *Ae. albopictus*, *Oc. triseriatus*, and *Cq. perturbans* based on a 3-way ANOVA. Significantly more *Ae. albopictus* were collected with octenol [P<.01]. This was also true for collections of *Oc. triseriatus* [P<.05] and *Cq. perturbans* [P<.01]. *Aedes albopictus* collections were increased 3.5 fold with octenol. *Ochlerotatus triseriatus* collections were increased 12 fold with use of octenol. Octenol increased *Cq. perturbans* collections by 20 fold.

Based on a Chi-square analysis of mosquito species trapped in low number, i.e., totals less than 100 specimens of *An. punctipennis*, *Cx. restuans*, *Cx. salinarius*, and *Ps. columbiae* were all significantly enhanced by use of octenol [P=.05].

The Proc Corr analysis comparing week, collections, and landing counts showed a significant correlation between collections and landing counts of *Ae. albopictus* [P<.01], (Figure 15). Since only *Ae. albopictus* was collected during the landing counts, no comparisons could be made between collections and week for other species trapped throughout the 8-week trial. Mean landing counts/ 2 minutes ranged from a high of 22 *Ae. albopictus* to a low of 4 *Ae. albopictus*. The overall landing-count average during the 8-week study was approximately 12 *Ae. albopictus* biting every two minutes.

A total of 1466 mosquito larvae, representing 7 species in 6 genera, was collected from tires at the salvage yard during the study period. They were, in decreasing order, *Ae. albopictus* (902, *Cx. territans* (289), *Oc. triseriatus* (211), *Or. signifera* (46), *Cx. restuans* (13), *Tx. rutilus* (3), and *Anopheles* species (2) (Table 12).

Discussion

Previous studies have shown that the combination of CO_2 and octenol significantly increased collections of *Cq. perturbans, An. punctipennis, Ps. columbiae, Cx. restuans,* and *Cx. salinarius* (Kline et al. 1990, Kline et al. 1991, Kline 1994, Rueda et al 2001). The results of the field trials in our study support these reports. The use of octenol with the MMP trap was effective in enhancing the response of many mosquito species, making it an effective trap for general monitoring of mosquito populations.

The results of preliminary field trials 1 and 2 indicated that there was a general trend of increased response of most species collected with the MMP and octenol, in that 75% of mosquito collections throughout the trials were trapped with octenol. Octenol

significantly enhanced collections of *Ae. albopictus* at Field Site 1 and 2. *Aedes vexans*, *An. punctipennis*, and *Cx. quinquefasciatus* collections were significantly enhanced at Field Site 2. Collections of these species at Field Site 1 were not statistically different. However, with the exception of *An. punctipennis*, these species were collected in greater numbers with octenol-baited traps. There was a negative effect of octenol observed in the collections of *An. punctipennis* in Field trail 1. Kline et al. (1991) found that octenol at times enhanced collections of anopheline mosquitoes and at other times appeared to repel these species. This observation is supported by the data reported here.

The mosquitoes trapped at Field Site 3 showed a positive response to the MMP baited with octenol, such that 81% of mosquito collections in this study were collected with octenol. *Aedes albopictus, Oc. triseriatus, Cq. perturbans, An. punctipennis, Ps. columbiae, Cx. restuans,* and *Cx. salinarius* showed a positive significant response to octenol-baited traps with overall collections increased 4 fold.

To our knowledge, this is the first published report of *Ae. albopictus* and *Oc. triseriatus* being significantly attracted to combinations of CO₂ and octenol. Since *Ae. albopictus* was trapped more during all 3 field trials with octenol, the previous claim that octenol is repellant to *Ae. albopictus* is not supported in this study. In fact in field trial 3, *Ae. albopictus* was trapped more with octenol throughout each week's trap rotation with a 3-fold increase in collection numbers throughout the study. The claim that *Ae. albopictus* is repelled by octenol may be attributed to the fact that most studies evaluating the Mosquito Magnet® traps baited with octenol were conducted in salt marshes where *Ae. albopictus* is not commonly collected. The studies reported here show that in an urban environment octenol does enhance collections of *Ae. albopictus* and is driving the response of *Ae. albopictus* collections.

Propane-powered traps did not reduce the natural populations of *Ae. albopictus* below the nuisance levels at field site 3. If the MMP reduced the natural populations of Ae. albopictus below nuisance levels a larger number of Ae. albopictus would have been collected in the MMP traps. Likewise a reduction in the numbers of *Ae. albopictus* landing during the landing counts would have been evident. This was not observed during field trial 3. There was a significant correlation between landing counts and MMP collections of Ae. albopictus. Even though the collections of Ae. albopictus in the MMP were high, Ae. albopictus biting activity was still considered to be above the nuisance level. Because our field sites were not typical of what homeowners face, it cannot be concluded that these traps would not reduce nuisance populations in a residential setting. If people live in an area where one or more mosquito species exhibit high numbers, the chances of reducing those populations to personally acceptable is low. In fact, Dennett et al.'s (2004) daily observations suggested that counterflow traps were efficient in not only capturing mosquitoes but also attracting mosquitoes that were never captured. Based on his observations, it is possible that the MMP trap attracts more mosquitoes than the trap actually captures, increasing overall mosquito abundance in the vicinity of the traps(s).

The results of this study indicated that octenol-baited MMP traps enhanced mosquito collections in all field trials. Because octenol causes a positive response in many species, the MMP trap can be an effective tool in monitoring mosquito populations. However, these traps alone may not be adequate for urban homeowners to control nuisance mosquitoes.

Literature Cited

- Anderson JR. 1989. Use of deer models to study larviposition by wild nasopharyngeal bot flies (Diptera: Oestridae). *J Med Entomol* 26:234-236.
- Burkett DA, Lee WJ, Lee KW, Kim HC, Lee HI, Lee JS, Shin EH, Wirtz RA, Cho HW, Claborn DM, Coleman RE, Klein TA. 2001. Light, carbon dioxide, and octenol-baited mosquito trap and host-seeking activity evaluations for mosquitoes in a malarious area of the republic of Korea. J Am Mosq Control Assoc 17:196-205.
- Dennett JA, Vessey NY, Parsons RE. 2004. A comparison of seven traps used for collection of *Aedes albopictus* and *Aedes aegypti* originating from a large tire repository in Harris County (Houston), Texas. J Am Mosq Control Assoc 20:342-349.
- French FE, Kline DL. 1989. 1-octen-3-ol, an effective attractant for Tabanidae (Diptera). *J Med Entomol* 26:459-461.
- Gillies, MT. 1980. The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bull Entomol Res* 70:525-532.
- Hall DR, Beevor PS, Cork A, Nesbitt BF, Vale GA. 1984. 1-octen-3-ol: a potent olfactory stimulant and attractant for tsetse isolated from cattle odors. *Insect Sci Appl* 5:335-339.
- Kemme JA, Van Essen PHA, Ritchie SA, Kayo BH. 1993. Response of mosquitoes to carbon dioxide and 1-octen-3-ol in southeast Queensland, Australia. *J Am Mosq Control Assoc* 9:431-435.
- Kline DL. 1994. Olfactory attractants for mosquito surveillance and control: 1-octen-3ol. J Am Mosq Control Assoc 10:280-287.
- Kline DL. 2002. Evaluation of various models of propane-powered mosquito traps. J Vect Ecol 1-7.

- Kline DL, Dame DA, Meisch MV. 1991. Evaluation of 1-octeno-3-ol and carbon dioxide as attractants for mosquitoes associated with irrigated rice fields in Arkansas. *J Am Mosq Control Assoc* 7: 165-169.
- Kline DL, Mann MO. 1998. Evaluation of butanone, carbons dioxide and 1-octen-3-ol as attractants for mosquitoes associated with North Central Florida Bay and cypress swamps. *J Am Mosq Control Assoc* 14:289-297.
- Kline DL, Takken W, Wood JR, Carlson DA. 1990. Field Studies on the potential of butanone, carbon dioxide, honey extract, 1-octen-3-ol, lactic acid, and phenols as attractants for mosquitoes. *Med Vet Entomol* 4:383-391.
- Mboera LE, Takken W, Sambu EZ. 2000. The response of Culex quinquefasciatus (Diptera: Culicidae) to traps baited with carbon dioxide, 1-octen-3-ol, acetone, butyric acid, and human foot odour in Tanzania. *Bull Entomol Res* 90:155-159.
- Rueda LM, Harrison BA, Brown JS, Whitt PB, Harrison RL, Gardner RC. 2001. Evaluation of 1-octen-3-ol, carbon dioxide, and light as attractants for mosquitoes associated with two distinct habitats in North Carolina. J Am Mosq Control Assoc 17:61-66.
- Shone SM, Ferrao PN, Lesser CR, Glass GE, Norris DE. 2003. Evaluation of carbon dioxide and 1-octen-3-ol-baited Centers for Disease Control Fay-Prince traps to collect *Aedes albopictus*. J Am Mosq Control Assoc 19:445-447.
- Takken W, Kline DL. 1989. Carbon dioxide and 1-octen-3-ol as mosquito attractants. *J Am Mosq Control Assoc* 5:311-316.
- Torr SJ. 1994. The tsetse (Diptera: Glossinidae) story: implications for mosquitoes. J Am Mosq Control Assoc 10:258-265.

	With Octenol	Without Octenol		
Mosquito Species	No. (%)	No. (%)		
Aedes albopictus	1051 (71)	421 (29)		
Anopheles punctipennis	0 (0)	14 (100)		
Culex quinquefasciatus	4 (57)	3 (43)		
Aedes vexans	2 (50)	2 (50)		
Culex restuans	2 (100)	0 (0)		
Psorophora columbiae	1 (100)	0 (0)		
Ochlerotatus triseriatus	1 (100)	0 (0)		
Totals	1061	440		

Table 6: Mosquito species collected with and without octenol at Field Site 1, June 2004. Species are listed in descending order of the total number of each species collected.

	Tre	atment	Treatme	ent x Ti	me	
Mosquito Species	Р	F	DF	Р	F	DF
Aedes albopictus	0.0321	29.64	1	0.5384	0.89	7

Table 7. Repeated measures ANOVA values for mosquito species with trap collections >100 specimens at Field Site 1.

	With Octenol	Without Octenol		
Mosquito Species	No. (%)	No. (%)		
Aedes albopictus	489 (86)	81 (14)		
Anopheles punctipennis	27 (77)	8 (23)		
Culex quinquefasciatus	17 (63)	10 (37)		
Aedes vexans	18 (95)	1 (5)		
Ochlerotatus triseriatus	0 (0)	2 (100)		
Psorophora columbiae	1 (50)	1 (50)		
Totals	552	103		

Table 8. Mosquito species collected with and without octenol at Field Site 2, June-July 2004. Species are listed in descending order of the total number of each species collected.

	Treatment			Treatment x Time		
Mosquito Species	Р	F	DF	Р	F	DF
Aedes albopictus	0.0280	34.24	1	0.0872	2.67	5

Table 9. Repeated measures ANOVA values for mosquito species with trap collections >100 specimens at Field Site 2.

	With Octenol		Without Oc	etenol
Mosquito Species	No.	(%)	No.	(%)
Aedes albopictus	4334	(78)	1237	(22)
Ochlerotatus triseriatus	1202	(92)	100	(8)
Coquillettida perturbans	125	(95)	6	(5)
Anopheles punctipennis	33	(94)	2	(6)
Psorophora columbiae	28	(85)	5	(15)
Culex restuans	20	(69)	9	(31)
Culex salinarius	13	(100)	0	(0)
Culex erraticus	8	(89)	1	(11)
Culex quinquefasciatus	1	(12)	7	(88)
Anopheles crucians	4	(80)	1	(20)
Aedes vexans	2	(67)	1	(33)
Psorophora ferox	2	(67)	1	(33)
Culex territans	1	(100)	0	(0)
Totals	5773		1370	

Table 10. Mosquito species collected with and without octenol at Field Site 3, July-August 2004. Species are listed in descending order of the total number of each species collected.

	Trea	tment		Treatm	ent x P	os	Treatm	ent x We	eek
Species	Р	F	DF	Р	F	DF	Р	F	DF
Ae. albopictus	0.0003	38.04	1	0.402	1.11	3	0.0114	6.1	6
Oc. triseriatus	0.0003	38.56	1	0.769	0.38	3	0.0004	16.13	6
Cq. perturbans	< 0.0001	51.06	1	0.4318	1.02	3	0.0541	3.47	6

Table 11. ANOVA values for mosquito species with trap collections > 100 specimens at Field Site 3.

Species	No.
Aedes albopictus	902
Culex territans	289
Ochlerotatus triseriatus	211
Orthopodomyia signifera	46
Culex restuans	13
Anopheles spp	2
Toxorhynchites rutilus	3
Totals	1466

Table 12. Mosquito larval samples collected from tires at parts-and-salvage yard in Phenix City, Alabama, during Field Trial 3 evaluating the Mosquito Magnet ProTM traps with and without octenol.



Figure 11. American Biophysics Corporation's propane-powered Mosquito Magnet Pro^{TM} trap.



Figure 12. Collections of landing mosquitoes using a hand-held, battery-operated aspirator to remove landing females during a 2-minute period to determine biting activity at a parts-and-salvage yard, in Phenix City, Alabama, 2004.



Figure 13. Collections of *Aedes albopictus* with and without octenol at Field Site 2 during successive trap nights. Collections of *Aedes albopictus* (black) were made with octenol. Collections of *Ae. albopictus* (gray) were made without octenol. Five trap-night collections were significantly enhanced with octenol (P < .05), as indicated by asterisks, based on a Chi-square analysis.



Figure 14. Collections of *Aedes albopictus* with and without octenol at Field Site 2 during successive trap nights. Collections of *Aedes albopictus* (black) were made with octenol. Collections of *Ae. albopictus* (gray) were made without octenol. Nine trap-night collections were significantly enhanced with octenol (P < .05), as indicated by asterisks, based on a Chi-square analysis.



Figure 15. MMP collections compared to mean landing counts of *Ae. albopictus* by trap week. MMP collections on the left y-axis and mean landing-count collections on right y-axis of *Aedes albopictus* at Field Site 3. Based on a Proc Corr analysis, the mean landing counts of *Ae. albopictus* were significantly correlated with the MMP collections by week, P < .05.

APPENDIX

List of mosquito species mentioned in the text and their author (Darsie and Ward 1981). Mosquito species are listed in alphabetical order.

Mosquito Species	Author
Aedes albopictus	(Skuse)
Aedes aegypti	(Linnaeus)
Aedes vexans	(Meigen)
Anopheles barberi	Coquillett
Anopheles crucians	Wiedemann
Anopheles punctipennis	(Say)
Anopheles quadrimaculatus	Say
Culex erraticus	(Dyar and Knab)
Culex pipiens	Linnaeus
Culex restuans	Theobald
Culex quinquefasciatus	Say
Culex salinarius	Coquillett
Culex territans	Walker
Coquillettida perturbans	(Walker)
Culiseta melanura	(Coquillett)
Ochlerotatus atropalpus	(Coquillett)
Ochlerotatus bahamensis	Berlin
Ochlerotatus japonicus	(Theobald)
Ochlerotatus sollicitans	(Walker)
Ochlerotatus taeniorhynchus	(Wiedmann)
Ochlerotatus triseriatus	(Say)
Orthopodomyia signifera	(Coquillett)
Mansonia titillans	(Walker)
Psorophora columbiae	(Dyar and Knab)
Psorophora ferox	(von Humboldt)
Toxorhvnchites rutilus	(Coquillett)