

## LARVAL SURVEY OF TIRE-BREEDING MOSQUITOES IN ALABAMA

WHITNEY A. QUALLS AND GARY R. MULLEN

Department of Entomology and Plant Pathology, 301 Funchess Hall, Auburn University, AL 36849-5413

**ABSTRACT.** A state-wide survey of tire-breeding mosquitoes in Alabama was conducted in 2004 and 2005. Tire sites in all 67 counties in the state of Alabama were sampled for mosquito larvae. A total of 13,022 mosquito larvae, representing 12 species in 7 genera, was collected. The most frequently collected species were *Aedes albopictus* (70.4%), *Culex territans* (8.0%), and *Ochlerotatus triseriatus* (7.1%). The following species were also collected: *Cx. restuans* (6.0%), *Cx. salinarius* (2.7%), *Orthopodomyia signifera* (2.4%), and *Cx. quinquefasciatus* (1.4%). *Ochlerotatus atropalpus*, *Toxorhynchites rutilus*, *Anopheles punctipennis*, *An. quadrimaculatus*, and *Psorophora columbiae* each represented <1.0% of the total larval collections. No *Ae. aegypti* or *Oc. japonicus* were collected from tires during this survey. The first known collection of *Ps. columbiae* breeding in water-filled tires is reported.

**KEY WORDS** *Aedes albopictus*, tires, *Aedes aegypti*, *Ochlerotatus japonicus*

### INTRODUCTION

Since the introduction of *Aedes albopictus* into North America via tire shipments from Asia in the early 1980s (Sprengr and Wuithiranyagool 1986), the significance of tires as an important mosquito development site has drawn increased attention by public health officials. Water-filled tires promote the proliferation and dispersal of mosquitoes, increasing the potential of public health problems ranging from nuisance complaints to transmission of mosquito-borne disease agents.

The accumulation of used tires in urban areas, along with the introduction of *Ae. albopictus*, has apparently changed the composition of tire-breeding mosquitoes in North America (Edgerly et al. 1993). *Aedes aegypti* was once the dominant mosquito breeding in tree holes and artificial containers, including tires, in Alabama. It has been reported, though, that there has been a noticeable decline in *Ae. aegypti* populations, most notably in some habitats in Florida (O'Meara et al. 1992), South Carolina (Richardson et al. 1995), and Louisiana (Nasci et al. 1989). In these reports, *Ae. albopictus* has been associated with the displacement of *Ae. aegypti*. Studies have also suggested that *Ae. albopictus* is involved in displacing other container breeders, such as *Ochlerotatus triseriatus* and *Oc. atropalpus* (Juliano 1998).

Another species of interest from an invasive-species viewpoint is *Oc. japonicus*. *Ochlerotatus japonicus* was first detected in the continental United States in New York in 1998. It is believed to have been introduced into the northeastern United States in used tires (Peyton et al. 1999). *Ochlerotatus japonicus* was detected within 3 years of its initial discovery in Connecticut, New Jersey, Pennsylvania, Ohio, Maryland, and Virginia. By the end of 2004 this species had extended its southern range into Georgia, North

Carolina (Gray et al. 2005), West Virginia (Joy and Sullivan 2005), and Alabama (Mullen 2005). What effect *Oc. japonicus* might have in displacing other tire-breeding mosquitoes or otherwise altering local abundance of tire-breeding species is uncertain.

No state-wide survey of tire-breeding mosquitoes in Alabama has been conducted since the first detection of *Ae. albopictus* at Cullman, AL, in 1986 (G. R. Mullen, unpublished data). Prior to that, *Ae. aegypti* was the dominant tire-breeding mosquito throughout the state. However, collections of *Ae. aegypti* in gravid traps, CDC light traps, and both tree holes and artificial containers have been scarce since that time.

The study reported here was conducted in 2004 and 2005 in cooperation with the Alabama Department of Public Health (ADPH). The purpose was to determine the geographic distributions of mosquito species utilizing tires as a larval habitat in Alabama, to determine relative frequencies of occurrence for those species utilizing tires as larval habitats, and to obtain current information on the presence or absence of *Ae. aegypti* and *Oc. japonicus* in the state.

### MATERIALS AND METHODS

With the assistance of ADPH area administrators and county environmentalists, discarded tires were sampled in all 67 Alabama counties over a 2-year period. Larval samples were collected from May 1 to October 31, 2004, followed by sampling tires in 2005 in those counties in which tire-breeding mosquitoes were not collected during 2004. The number of tires per site ranged from as few as 2 tires to over 50,000 tires. In 2004, 2 or more tire sites per county were sampled twice each month, with 1 collection being taken during the first half of the month and the second collection during the latter

half of the month. In 2005, only 1 or 2 tire sites were sampled from each of the previously unsampled counties in 2005.

Each ADPH county environmentalist was provided with a larval sampling kit that included a baster, hand-held dipper, a 0.40-mm fine-mesh aquarium net, a 16-fl-oz bottle of 70% isopropyl rubbing alcohol, 12 4-dram vials, small-tip transfer pipettes, labels, and a 4-oz container for alcohol. They were also given a written protocol instructing them to: 1) collect a larval sample with either the dipper or baster, 2) pour the sample through the aquarium net, 3) invert the aquarium net into the container of alcohol, and (4) pipette the larvae from the container into 4-dram vials filled with alcohol. All samples were labeled with the county, location, and date. At least 4 tires were sampled from each tire site, with a minimum total collection of 20 larvae per collection site.

Larvae were sent to the medical entomology laboratory at Auburn University via the ADPH courier system for identification. All 3rd- and 4th-stage larvae were identified, whereas 1st- and 2nd-stage larvae and pupae were disregarded during this survey.

## RESULTS

Tires were sampled in 52 of Alabama's 67 counties during the 6-month period from May 1 to October 31, 2004 (Fig. 1). The remaining 15 counties subsequently sampled in 2005 were Cherokee, Coosa, Houston, Jefferson, Lamar, Lawrence, Limestone, Madison, Marengo, Perry, Randolph, Tallapoosa, Walker, Wilcox, and Winston. These counties were sampled by the authors.

A total of 169 tire sites was sampled during this survey, with a total of 13,022 mosquito larvae, representing 12 species in 7 genera, being identified (Table 1). The most frequently collected species were *Ae. albopictus*, *Culex territans*, and *Oc. triseriatus*. The following species were also collected: *Cx. restuans*, *Cx. salinarius*, *Orthopodomyia signifera*, and *Cx. quinquefasciatus*, with each of the following taxa representing < 1% of the total collections: *Oc. atropalpus*, *Toxorhynchites rutilus*, *Anopheles punctipennis*, *An. quadrimaculatus*, and *Psorophora columbiae*. The geographic distribution of each mosquito species collected from tires sites in this survey by county is presented in Figs. 2–4. The seasonal occurrence of each species arranged by total occurrence in collections during the first and second half of each collection period throughout the 2-year survey is shown in Fig. 5.

*Aedes albopictus*, *Cx. territans*, *Oc. triseriatus*, and *Cx. restuans* were present in collections throughout the season from the second half of May to the first week of October. *Aedes albopictus* was the only species collected in the

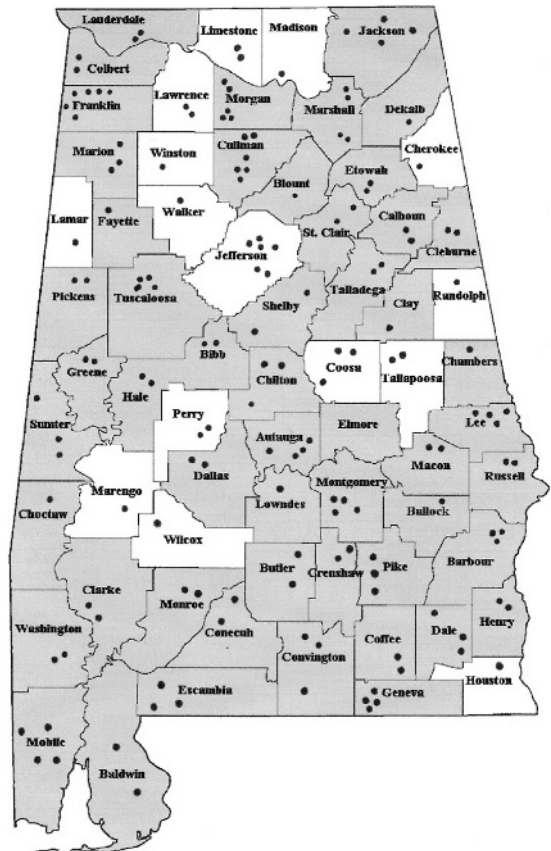


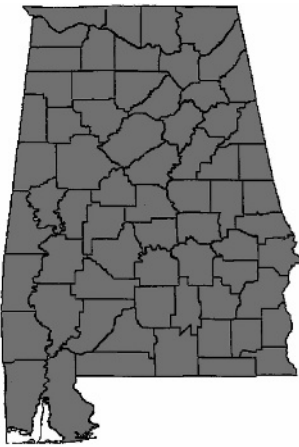
Fig. 1. Alabama counties in which larvae were collected from tires during 2-year survey. Counties shaded in gray, 2004; unshaded counties, 2005. Black dots represent locations of tire sites sampled in each county during the 2004–2005 larval survey. A total of 169 tire sites were sampled.

second half of October. *Culex salinarius* and *Cx. quinquefasciatus* were collected at least once during each month during the larval survey period. *Orthopodomyia signifera* was consistently collected each month except May. For species that were collected less frequently, seasonal differences were observed in larval collections. 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.

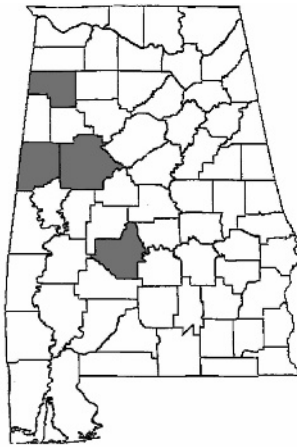
*Aedes albopictus* co-occurred in tire yards with *Oc. triseriatus* in 39 of the 67 counties. No *Ae. aegypti* larvae were found in tires during this study, nor were larvae of the recently introduced *Oc. japonicus*. It should be noted that *Oc. japonicus* was not detected in Alabama until June

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.

Mosquito species	May	June	July	August	September	October	Totals (%)
<i>Aedes albopictus</i>	129	1832	3234	2088	1451	429	9163 (70.4)
<i>Culex territans</i>	29	135	171	296	220	192	1043 (8.0)
<i>Ochlerotatus triseriatus</i>	0	190	370	195	148	18	921 (7.1)
<i>Culex restuans</i>	44	393	94	156	62	28	777 (6.0)
<i>Culex salinarius</i>	15	58	121	61	85	13	353 (2.7)
<i>Orthopodomyia signifera</i>	0	51	132	32	73	28	316 (2.4)
<i>Culex quinquefasciatus</i>	3	9	27	8	93	36	176 (1.4)
<i>Ochlerotatus atropalpus</i>	0	35	39	30	29	0	133 (1.0)
<i>Toxorhynchites rutilus</i>	0	14	28	22	19	0	83 (0.6)
<i>Psorophora columbiae</i>	0	13	8	0	0	0	21 (0.2)
<i>Anopheles punctipennis</i>	0	10	0	0	0	0	10 (<0.1)
<i>Anopheles quadrimaculatus</i>	0	3	0	0	0	0	3 (<0.1)
Unidentified <i>Anopheles</i> spp.	1	3	10	8	0	1	23 (0.2)
Totals	221	2746	4234	2896	2180	745	13022



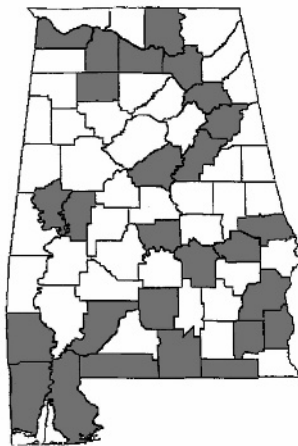
*Aedes albopictus*



*Culex quinquefasciatus*



*Culex restuans*



*Culex salinarius*

Fig. 2. Distribution, by county, of tire-breeding mosquitoes in Alabama, based on 2004–2005 larval survey. *Aedes albopictus*, *Culex quinquefasciatus*, *Cx. restuans*, and *Cx. salinarius*.

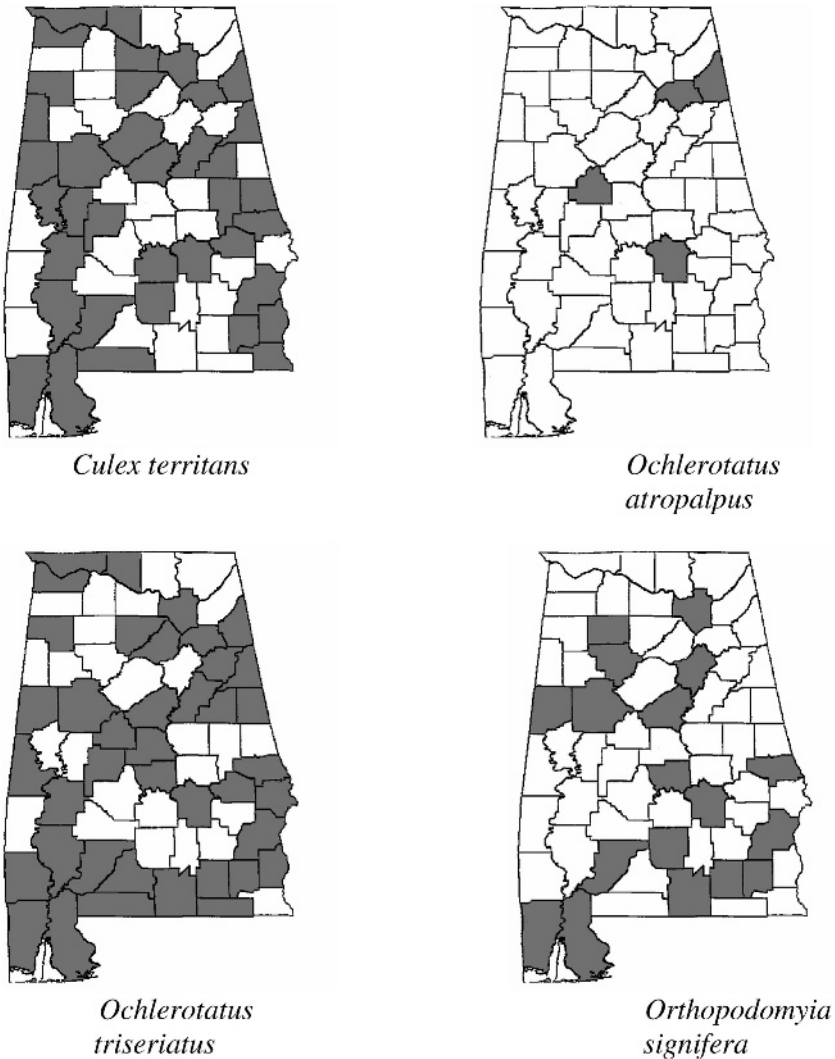


Fig. 3. Distribution, by county, of tire-breeding mosquitoes in Alabama, based on 2004–2005 larval survey. *Culex territans*, *Ochlerotatus atropalpus*, *Oc. triseriatus*, and *Orthopodomyia signifera*.

21, 2005, and then only as adults in Jackson County in the extreme northeastern corner of the state (Mullen 2005).

### DISCUSSION

This study shows that at least 12 mosquito species utilize tires as a larval habitat in Alabama. *Aedes albopictus* is by far the most common, representing more than 70% of the total larvae collected from tires. It was collected from all 169 tire sites sampled, documenting its presence in every county in Alabama. The 3 commonly occurring species (>5%) in tires were *Cx. territans*, *Oc. triseriatus*, and *Cx. restuans*. Less frequently (<5%) collected species included *Cx. salinarius*, *Or. signifera*, and *Cx. quinquefasciatus*.

*Ochlerotatus atropalpus*, *Tx. rutilus*, *An. punctipennis*, *An. quadrimaculatus*, and *Ps. columbiae* collections were considered to be incidental, with collections representing less than 1%.

Because *Ae. aegypti* was not collected at all in this survey, the implication is that *Ae. albopictus* has virtually displaced *Ae. aegypti* as a tire breeder in Alabama. *Aedes albopictus* was collected in association with the 12 other mosquito species at tire sites in the survey.

*Culex territans* was collected from 103 tire sites during this survey co-occurring with collections of *Cx. restuans* 85% of the time, *Cx. quinquefasciatus* 40% of the time, and *Oc. triseriatus* 25% of the time. Although previous studies have reported that *Cx. territans* does not commonly utilize tires as a larval habitat (Wilmot et al. 1992,

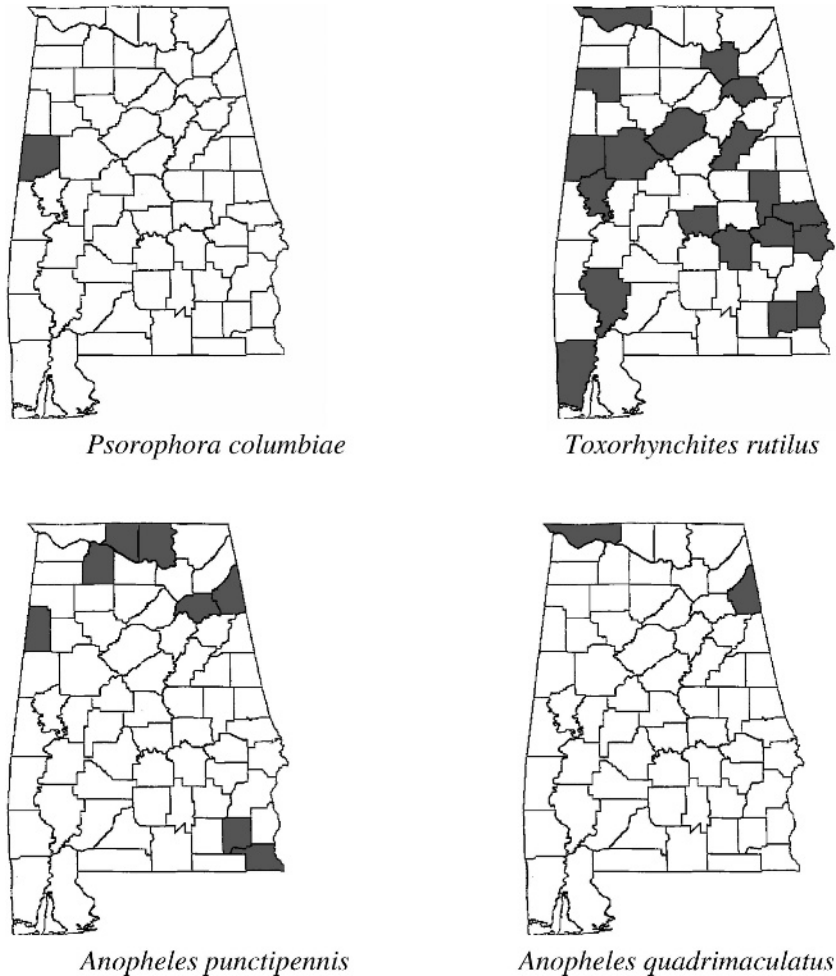


Fig. 4. Distribution, by county, of tire-breeding mosquitoes in Alabama, based on 2004–2005 larval survey. *Psorophora columbiae*, *Toxorhynchites rutilus*, *Anopheles punctipennis*, and *An. quadrimaculatus*.

Jamieson et al. 1994), a relatively high prevalence of *Cx. territans* larvae in tires has been documented in West Virginia (Joy et al. 2003). Our data reflect a situation similar to that in West Virginia in that *Cx. territans* was collected at 103 out of 169 tire sites, an overall prevalence of 61%. This prevalence corresponds favorably with West Virginia's findings in certain months.

*Ochlerotatus triseriatus* was collected from 120 tire sites and co-occurred with *Ae. albopictus* in 39 of the 67 counties sampled 30% of the time. *Ochlerotatus triseriatus* was collected from June to October. 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) and Swason et al. (2000) suggested that temporal and spatial differences between these 2 species would decrease the likelihood of *Oc. triseriatus* being displaced by

*Ae. albopictus*. Joy and Sullivan (2005) found *Ae. albopictus* to compete effectively with *Oc. triseriatus* from midsummer through October in West Virginia counties with low elevations, but was not well represented in counties with higher elevations, corroborating the previous suggestion. The data in this survey indicated that many natural container breeders like *Oc. triseriatus* and *Or. signifera* commonly oviposited in tires at the same sites where *Ae. albopictus* is the dominant species.

*Culex restuans* was collected from 32 tire sites in 15 counties during the survey. Berry and Craig (1984) observed a peak of *Cx. restuans* populations during mid-June, with *Cx. restuans* being replaced by *Cx. pipiens* during July in the midwestern United States. In Alabama, however, *Cx. restuans* showed only a slight decline after July based on larval collections from tires. The latter also was observed in populations of *Cx. restuans* from tire collections in a tire-breeding

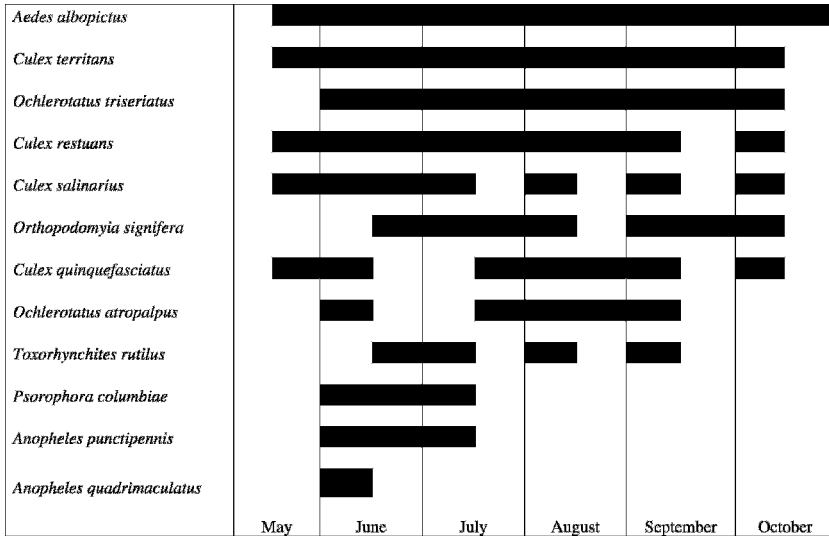


Fig. 5. Presence or absence of tire-breeding mosquitoes in Alabama, based on 2-week intervals from May through October, are listed in descending order of their seasonal occurrence throughout the larval survey period.

mosquito survey in 9 used-tire disposal sites in Connecticut (Andreadis 1988).

*Culex salinarius* was collected from 38 tire sites in 27 counties during this survey. The prevalence of this species in a study in Illinois was so low that little can be said about its distribution and abundance or seasonal changes (Lampman et al. 1997). Although peaks during the first week of July and September were observed in the larval survey, no conclusions about its abundance or seasonality can be determined.

*Orthopodomyia signifera* was collected in 18 counties from 22 tire sites, with the first occurrence of this species in late June from Coffee County, AL, in the southeastern part of the state. This species was found in combination with *Ae. albopictus* 90% of the time and with *Oc. triseriatus* about 37% of the time. *Orthopodomyia signifera* is a common tree hole breeder and has a preference for shaded tires (Lampman et al. 1997).

*Culex quinquefasciatus* was collected from 5 tire sites in 4 counties from May to October, with a peak in September. This species was usually found in association with the other *Culex* species collected during this survey.

The geographic occurrence of 11 of the 12 mosquito species collected in this survey fall within their previously known ranges (Darsie and Ward 2005). However, the exception is *Oc. atropalpus* collected in Montgomery County, farther south than its previously documented occurrence in the Tennessee Valley area of Alabama (Darsie and Ward 2005). *Ochlerotatus atropalpus* was collected from only 1 tire site at Lamuck, AL (32°20.613'N, 86°21.285'W) on July

2, 2004. 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 Karsnick 1970), they have the potential to exploit large numbers of tires, in turn extending their distribution (Beier et al. 1983). 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 another area where *Oc. atropalpus* is already established.

*Toxorhynchites rutilus* was collected from 28 tire sites in 19 counties. This species was collected from the second half of June to mid-September. Although *Tx. rutilus* was frequently collected from tires also in later months in West Virginia (Joy et al. 2003, Joy and Sullivan 2005), it was not a species commonly found developing in tires in Alabama.

During this survey very few *Anopheles* larvae were collected. *Anopheles punctipennis* and *An. quadrimaculatus* were collected in 2005 during the collection trips made to finish up the larval survey. The *Anopheles* larvae collected from tires in 2004 were early instars and were not identified species. *Anopheles* collections were primarily in the early summer months, with few to no collections after late July.

*Psorophora columbiae* was collected from only 1 tire site, in Pickens County in west-central Alabama bordering Mississippi (33°6.946'N, 88°10.165'N) on June 17 and 28 and July 7, 2004. This species usually breeds in woodland

pools and previously has not been reported developing 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* during this survey, nor had this species been reported in Alabama prior to this survey. The previously reported southernmost collection of *Oc. japonicus* was in Fulton County, GA (Gray et al. 2005). However, in the summer of 2005 a single adult *Oc. japonicus* female was collected by Kristy Gottfried (Tennessee Valley Authority, Muscle Shoals, AL) in a gravid trap in Jackson County, AL, in the extreme northeastern corner of the state (Mullen 2005). *Aedes albopictus* was the only mosquito species collected as larvae at tire sites in Jackson County during this survey.

Statewide surveys provide baseline data for future documentation of newly introduced species and their subsequent geographic spread. As more mosquito species are introduced into the Americas through international transportation, it is important to have current data on the geographic distributions of the already established mosquito fauna as provided by the Alabama survey reported here.

#### ACKNOWLEDGMENTS

This research was conducted with the support of the Alabama Department of Public Health. We wish to thank the following county ADPH personnel who provided field assistance or guidance during this research: A. Salter (Autauga), Hicks (Barbour), J. Kelly (Blount), R. B. Newton (Butler), E. B. Haskew (Calhoun), G. Adams (Chambers), B. Robinson (Choctaw), K. Bush (Clarke), R. Stinson (Cleburne), W. Edberg and D. Dean (Coffee), A. Cosby (Colbert), Henley and Williamson (Covington), J. Cowen (Crenshaw), B. Stephens and P. Lindsey (Cullman), G. Malkoff (Dale), K. Hope (DeKalb), L. Salter (Elmore), D. Hulsey (Etowah), P. Wright (Franklin), J. Hughes (Geneva), Thompson (Greene), Wilson (Hale), Bell and Phillips (Henry), C. Harmon and B. Niemeyer (Lee), Medley and LeMaster (Marion), Stephens and Ball (Marshall), G. Kohr (Mobile), Lynn Smith (Montgomery), M. Cassidy (Morgan), B. Ambrose (Pickens), L. Williford (Pike), Burrell (Russell), T. Young (St. Clair), B. Banks (Shelby), B. Webb (Sumter), H. Peters (Talladega), T. Dockery (Tuscaloosa), Bush (Washington), and the other ADPH county environmentalists who provided assistance during this research. We also wish to thank Ashley Lovell (USDA-Aphis). We appreciated the field assistance provided by Scott Croxton, undergraduate research assistant and Nathan Burkett, graduate

research assistant. This work was supported in part by the Centers for Disease Control and Prevention, with funds administered through the Alabama Department of Public Health.

#### REFERENCES CITED

- Andreadis TG. 1988. A survey of mosquitoes breeding in used tire stockpiles in Connecticut. *J Am Control Assoc* 4:256–260.
- 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.
- Berry WJ, Craig GB Jr. 1984. Bionomics of *Aedes atropalpus* breeding in scrap tires in northern Indiana. *Mosq News* 44:476–484.
- Craig GB Jr. 1980. *Aedes atropalpus* introduced into Indiana: is this mosquito a potential vector of arboviruses? *Proc Indiana Vector Control Assoc* 4:3–7.
- 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.
- Egerly JS, Willey MS, Livdahl TP. 1993. The community ecology of *Aedes* egg hatching: implications for a mosquito invasion. *Ecol Entomol* 18:123–128.
- 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.
- 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.
- 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.
- Joy JE, Sullivan SN. 2005. Occurrence of tire inhabiting mosquito larvae in different geographic regions of West Virginia. *J Am Mosq Control Assoc* 21:380–386.
- Juliano SA. 1998. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? *Ecology* 79:255–268.
- Lampman R, 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.
- Mullen GR. 2005. First report of *Ochlerotatus japonicus* in Alabama. *Ala Vector Manage Soc Newsl* 15(2):2.
- Nasci RS, Hare SG, Willis FS. 1989. Interspecific mating between Louisiana strains of *Aedes albopictus* and *Aedes aegypti* in the field and laboratory. *J Am Mosq Control Assoc* 5:416–421.
- 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.

- O'Meara GF, 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.
- Peyton EL, Campbell SR, Candeletti TM, Romanowski M, Crans W. 1999. *Aedes* (Finlaya) *japonicus japonicus* (Theobald), a new introduction into the United States. *J Am Mosq Control Assoc* 15:238-241.
- Richardson JH, Barton WE, Williams DC. 1995. Survey of container-inhabiting mosquitoes in Clemson, South Carolina, with emphasis on *Aedes albopictus*. *J Am Mosq Control Assoc* 11:396-400.
- Sprenger D, Wuithiranyagool T. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *J Am Mosq Control Assoc* 2:217-218.
- Swason J, Lancaster M, Anderson J, Crandell M, Haraims L, Grimstad P, Kitron U. 2000. Overwintering and establishment of *Aedes albopictus* (Diptera: Culicidae) in an Urban La Crosse Virus enzootic site in Illinois. *J Med Entomol* 37:454-460.
- 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.