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LARVAL MOSQUITOES IN ABANDONED TIRE PILE SITES FROM WEST VIRGINIA

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ABSTRACT. Larvae of 13 mosquito species were collected from abandoned tire piles at peridomestic and nonperidomestic sites in 3 south-central West Virginia counties from May through September 2002. *Ochlerotatus triseriatus* was the most frequently collected species from May through August, whereas *Aedes albopictus* and *Ochlerotatus japonicus* were more prevalent in September. Prevalence of *Oc. triseriatus* and *Culex restuans* declined throughout the study period. Conversely, prevalences of *Ae. albopictus, Oc. japonicus, Culex territans,* and *Toxorhynchites rutilus* increased during the same period. *Ochlerotatus atropalpus* was significantly more likely to be encountered at nonperidomestic sites. None of the other species exhibited a significant predisposition for either peridomestic or nonperidomestic sites.

KEY WORDS Arboviruses, *Ochlerotatus triseriatus, Ochlerotatus japonicus, Aedes albopictus,* invasive species

INTRODUCTION

Nasci et al. (2000) acknowledged that the influence of habitat on the transmission cycle of the La Crosse encephalitis virus (LAC) was not fully understood. Work by those investigators in Nicholas County, West Virginia, in 1996 indicated that La Crosse encephalitis cases and the presence of LAC in *Ochlerotatus triseriatus* (Say) were associated with a variety of habitat types, leading to the conclusion that the dynamics of enzootic LAC transmission by this vector species were very complex. Ultimately, those investigators found no clear set of habitat parameters that could be used as predictors for the presence or absence of LAC in a particular area of Nicholas County.

Two Asian mosquito species have been introduced into West Virginia since that 1996 study by Nasci et al. (2000): *Aedes albopictus* (Skuse) in 1998, and *Ochlerotatus japonicus* (Theobald) in 2002. Introductions of these exotic species may have made the dynamics of LAC transmission even more complex for 2 reasons. First, the Asian species may compete with *Oc. triseriatus* for space and food resources, thus altering—in unknown ways—population structure of the native LAC vector. The introduction of *Oc. japonicus* is too recent to have produced any relevant data on this subject, but *Ae. albopictus* is thought by some workers (Ho et al. 1989, Livdahl and Willey 1991, Edgerly et al. 1993) to be capable of competing effectively with *Oc. triseriatus* and perhaps even displacing over the long-term. Second, with the isolation of LAC from naturally infected *Ae. albopictus* in Tennessee, Gerhardt et al. (2001) established that the Asian tiger mosquito may serve as an accessory LAC vector. The possibility that *Oc. japonicus* might become involved in LAC transmission as well cannot be ruled out.

So, from the standpoint of both a public health perspective and an investigation of competitive pressures that introduced species may bring to bear on native forms, the confluence of *Ae. albopictus, Oc. japonicus,* and *Oc. triseriatus* in West Virginia at this time highlights the desirability of continued surveillance activity on these 3 species.

Nicholas County was once considered the endemic focus for LAC, but Fayette and Raleigh counties, located immediately to the south of Nicholas County, are now experiencing comparable numbers of cases annually (West Virginia Department of Health and Human Resources, unpublished data 2002). Joy et al. (2003) reported on monthly surveillance results of tire-breeding species in Nicholas County based on collections made in 2001, but as a result of the more recent LAC reporting data in Fayette and Raleigh counties, the focus of the present surveillance work has been extended geographically to those latter counties.

MATERIALS AND METHODS

Study area: Nicholas, Fayette, and Raleigh counties, with a combined population of 153,000, are located in south-central West Virginia approximately between latitudes 37°30′ and 38°30′N and longitudes 80°30′ and 81°30′W.

Site characterization and collection procedure: A total of 112 abandoned tire pile sites were identified in the 3-county target area from May through September 2002. Each of those sites was placed into 1 of 2 categories (peridomestic or nonperidomestic) based on the proximity of the tire habitat to human hosts. Fifty-seven peridomestic sites had tires located in areas where humans were readily available to mosquitoes. These sites were businesses that offered automobile tire replacement services and were located within 100 m of homes, motorcycle or go-cart race tracks, and convenience stores. Additionally, 55 nonperidomestic sites (i.e., sites ≥100 m distant from areas of human activity) were examined. These sites were generally along rural roads where illegal dumping occurred. Tire-

Table 1. Frequencies (percent of visits positive) of larval mosquito species colonizing waste tire piles in south-central West Virginia (e.g., larval *Aedes albopictus* were collected from 123, or 36.6%, of the 336 total site visits). Number of visits is given in parentheses.

Species	Collection site visits Total (336)	Peridomestic (167)	Nonperidomestic (169)	χ^2 (1 df)
Aedes albopictus (Skuse)	36.6	32.9	40.2	1.628
Anopheles barberi (Coquillett)	1.2	0	2.4	—
Anopheles punctipennis (Say)	3.9	2.4	5.3	1.231
Anopheles quadrimaculatus Say	1.5	1.2	1.8	—
Culex pipiens Linnaeus	6.0	6.6	5.3	0.163
Culex restuans Theobald	43.2	46.1	40.2	0.953
Culex territans Walker	27.1	30.5	23.7	1.675
Ochlerotatus atropalpus (Coquillett)	17.0	11.4	23.1	6.056[1]
Ochlerotatus canadensis (Theobald)	5.4	4.8	5.9	0.047
Ochlerotatus japonicus (Theobald)	31.5	35.3	27.8	1.864
Ochlerotatus triseriatus (Say)	62.5	57.5	67.5	3.150
Orthopodomyia signifera (Coquillett)	2.4	1.8	3.0	—
Toxorhynchites rutilus septentrionalis (Coquillett)	19.6	19.8	19.5	0.007

[1] $P < 0.05$.

inhabiting mosquitoes at nonperidomestic sites did not have ready access to humans as a blood-meal source. Many sites were visited on more than 1 occasion, but no site was visited more than once in the same month.

Tires were sampled by repeatedly sweeping a handheld strainer with a mesh size of approximately 1.0 mm through the water and leafy substrate. Larvae collected in the strainer were removed with jeweler's forceps and placed in 30-ml bottles, appropriately labeled with a site number and date of collection, containing 95% ethanol. Larvae were identified with the aid of a Zeiss stereomicroscope at 60×. If identification was questionable, larvae were dehydrated to 99% ethanol, cleared in xylene, and mounted in Permount® (Fisher Scientific, Pittsburgh, PA) for examination under a compound microscope at greater magnification. Keys by Means (1979), Darsie and Ward (1981), and Darsie (1986) were consulted for larval identifications. In addition, *Oc. japonicus* was identified from the key made available by Rutgers University (2003).

Data analysis: A chi-square 2 × 2 table was used to test for statistical significance for larval species at peridomestic vs. nonperidomestic sites, whereas the chi-square test of equality of proportions (SAS Institute 1989) was employed to evaluate temporal distribution for each of the 7 most commonly encountered species. Levels of probability are given in the narrative or tables as appropriate.

RESULTS

General findings

Thirteen species of mosquito larvae were identified in 336 visits (167 and 169 visits to peridomestic and nonperidomestic sites, respectively) to 112 tire dump sites in Fayette, Nicholas, and Raleigh counties of West Virginia from May through September 2002 (Table 1). Overall frequency of occurrence was high (>50% of the site visits) for *Oc. triseriatus*; moderate (>20–50%) for *Culex restuans* (Theobald), *Ae. albopictus, Oc. japonicus,* and *Culex territans* (Walker); low (>5–20%) for *Toxorhynchites rutilus* (Coquillet), *Ochlerotatus atropalpus* (Coquillet), *Culex pipiens* L., and *Ochlerotatus canadensis* (Theobald); and rare (<5%) for *Anopheles punctipennis* (say), *Orthopodomyia signifera* (Coquillet), *Anopheles quadrimaculatus* Say, and *Anopheles barberi* (Coquillet).

Larval presence by site category

Larval colonization frequency patterns by site category varied according to species, with 5 species more often found at peridomestic sites, and 8 at nonperidomestic sites. However, no species was significantly more likely to be encountered at peridomestic sites, and only a single species, *Oc. atropalpus,* was significantly more likely to be observed at nonperidomestic tire habitats (Table 1).

Larval presence by month of collection

Ochlerotatus triseriatus was the most frequently collected species from May through August (Table 2). Both *Ae. albopictus* and *Oc. japonicus* were more frequently collected than *Oc. triseriatus* in September, but only the higher prevalence of 65.7% for *Ae. albopictus* (vs. 40.0% for *Oc. triseriatus*) was significant ($\chi^2 = 8.284$; 1 df; $P < 0.05$).

Prevalence (i.e., percent of visits positive in Table 2) of 2 species, *Oc. triseriatus* and *Cx. restuans,* gradually declined throughout the study period, whereas the reverse was true for *Ae. albopictus, Oc.*

Table 2. Larval distribution, by month of collection, for the 7 species in tire pile habitats where valid chi-square tests can be made. The negative (Neg.) and positive (Pos.) visits for each species total to the number of visits (shown in parentheses) for each month (total collections, all months = 336). The null hypothesis is that the proportion of positives is the same for each month for any given species. The null hypothesis is rejected for every species except *Ochlerotatus atropalpus*.[1]

	Oc. triseriatus		*Cx. restuans*		*Ae. albopictus*	
Month	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
May	9	49	23	35	50	8
(58)	15.5	84.5	39.7	60.3	86.2	13.8
	7.47	4.49	3.02	3.97	4.76	8.25
June	26	46	30	42	63	9
(72)	36.1	63.9	41.7	58.3	87.5	12.5
	0.04	0.02	2.92	3.84	6.60	11.43
July	23	43	33	33	40	26
(66)	34.8	65.2	50.0	50.0	60.6	39.4
	0.12	0.07	0.54	0.72	0.08	0.14
Aug.	26	44	54	16	36	34
(70)	37.1	62.9	77.1	22.9	51.4	48.6
	0.00	0.00	5.07	6.69	1.58	2.74
Sept.	42	28	51	19	24	46
(70)	60.0	40.0	72.9	27.1	34.3	65.7
	9.45	5.67	3.16	4.16	9.36	16.20
(336)	126	210	191	145	213	123
χ^2	27.34; df = 4; $P < 0.0001$		34.08; df = 4; $P < 0.0001$		61.13; df = 4; $P < 0.0001$	

[1] *Oc., Ochlerotatus*; *Cx., Culex*; *Ae., Aedes*; *Tx., Toxorhynchites.* Numbers in each of the 35 cells (as depicted in the 1st cell) represent (from top to bottom): number of visits negative or positive for mosquito larvae, percent of visits negative or positive for larvae, and negative or positive cell contribution to the total chi-square value. Note: 7 mosquito species × 5 monthly categories = 35 cells, with each cell having a negative and positive component.

japonicus, Cx. territans, and *Tx. rutilus.* Prevalence of *Oc. atropalpus* was relatively consistent over the collection period. Collections of *Cx. pipiens, Oc. canadensis,* and *Or. signifiera,* as well as the 3 anopheline species, were too few to determine monthly distributions.

DISCUSSION

The 13 species of mosquitoes using abandoned tires as larval habitats in 3 counties of south-central West Virginia compares favorably with the 12 species reported from tires in West Virginia (Joy et al. 2003), the 11 species reported from containers (mostly tires) in North Carolina (Szumlas et al. 1996), and the 14 species collected from tires in Michigan (Wilmot et al. 1992) and Illinois (Novak 1995). *Ochlerotatus japonicus* was 1st reported in West Virginia early in 2002 (James Amrine, personal communication), and this introduced species was well represented in our 2002 summer collections (Table 1).

Ochlerotatus triseriatus was the dominant species encountered in all months except September, when it was supplanted by both *Ae. albopictus* and *Oc. japonicus.* However, only the higher prevalence observed for *Ae. albopictus* vis-à-vis *Oc. triseriatus* in September was significant. This was in contrast to the results of Joy et al. (2003), who noted that prevalence of *Ae. albopictus* never reached levels attained by *Oc. triseriatus* in later months of 2001.

The possibility that resident populations of *Oc. triseriatus* could be displaced by the invasive *Ae. albopictus* has been suggested by several investigators (Ho et al. 1989, Livdahl and Willey 1991, Edgerly et al. 1993), although Livdahl and Willey (1991) cautioned that the predatory *Tx. rutilus* could influence the outcome of any *Ae. albopictus* invasion. Moore (1999) also questioned the ability of the Asian tiger mosquito to compete with resident species in natural conditions, citing the relative scarcity of *Ae. albopictus* in the northeastern United States. Another impediment to the displacement of *Oc. triseriatus* by *Ae. albopictus* is that the former species exhibits a superior cold hardiness over the latter (Hanson and Craig 1995). Evidence for this cold tolerance is seen in the very high prevalence of *Oc. triseriatus* in April and May (Joy et al. 2003), a phenomenon supported by the present study where the chi-square cell contribution for larval presence of *Oc. triseriatus* in May alone made up 44% of the total chi-square value (Table 2). Conversely, larval collections of *Ae. albopictus* were highest in September, with the chi-square cell contribution for that 1 month contributing approximately 42% of the total chi-square value (Table 2). These findings are in agreement with those of Lampman et al. (1997), who noted that *Ae. albopictus* was a major late-season species. Although examination of our data (Table 2) indicates that *Ae. albopictus* may be present in higher frequencies (in northeastern states such as West Virginia) than pre-

Table 2. Extended.

Oc. japonicus		*Cx. territans*		*Tx. rutilus*		*Oc. atropalpus*	
Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
58	0	50	8	58	0	52	6
100	0	86.2	13.8	100	0	89.7	10.3
8.43	18.30	1.41	3.78	2.79	11.4	0.31	1.50
53	19	55	17	67	5	56	16
73.6	26.4	76.4	23.6	93.1	6.9	77.8	22.2
0.28	0.61	0.12	0.32	1.45	5.91	0.24	1.17
37	29	44	22	55	11	52	14
56.1	43.9	66.7	33.3	83.3	16.7	78.8	21.2
1.48	3.21	0.35	0.95	0.07	0.30	0.14	0.70
41	29	52	18	44	26	57	13
58.6	41.4	74.3	25.7	62.9	37.1	81.5	18.3
1.00	2.17	0.02	0.05	2.67	10.91	0.02	0.11
41	29	44	26	46	24	62	8
58.6	41.4	62.9	37.1	65.7	34.3	88.6	11.4
1.00	2.17	0.97	2.62	1.87	7.64	0.26	1.27
230	106	245	91	270	66	279	57
38.64; df = 4; $P < 0.0001$		10.59; df = 4; $P = 0.0316$		44.99; df = 4; $P < 0.0001$		5.71; df = 4; $P = 0.2216$	

viously thought, this species does not overlap temporally with the resident *Oc. triseriatus.*

The monthly occurrence pattern for *Oc. japonicus* was more closely related to that of *Ae. albopictus* than *Oc. triseriatus* (Table 2), but because the bionomics of *Oc. japonicus* in the USA are largely unknown (Sardelis and Turell 2001), and this species is such a recent introduction to West Virginia, evaluation of its monthly distribution is premature. If *Oc. japonicus* becomes well established in West Virginia, it could be a significant competitor with *Oc. triseriatus* because it has a Palearctic distribution (Peyton et al. 1999) and has been reported as numerous in the early spring (Kamimura 1968). The public health importance of *Oc. japonicus* in the USA has not been studied in detail, but *Oc. japonicus* is an efficient laboratory vector of West Nile virus (WN), and may serve as an ideal bridge vector between birds and horses or humans (Turell et al. 2001).

Continued monitoring of the 2 introduced Asian species is desirable for 2 reasons: 1st, we are presented with an opportunity to study the effect(s) of invasive species on resident forms; and 2nd, the Asian species represent potential new vector opportunities for viral pathogens. The latter issue is particularly relevant to public health officials in West Virginia because LAC is highly endemic to the state (McJunkin et al. 2001), and LAC has been found in wild populations of *Ae. albopictus* (Gerhardt et al. 2001). These latter workers speculated that the continued range extension of *Ae. albopictus* could result in an increase in both incidence and distribution of LAC in the southeastern United States. The peak in recorded La Crosse encephalitis cases (1995–2001) for West Virginia occurs in August (West Virginia Department of Health and Human Services, unpublished data); thus, it could be postulated that the temporal extension of *Ae. albopictus* evident in this study could result in the increased incidence and distribution of La Crosse encephalitis cases as well. Such a proposition is strengthened by the findings of Grimstad et al. (1989), who, in working on LAC-competent vectors under laboratory conditions, concluded that introduced *Ae. albopictus* populations: ". . . must be considered potentially able to transmit LACV as efficiently as fresh *Ae. triseriatus* field populations."

A monthly decline in larval populations of *Oc. triseriatus* in West Virginia has been observed by Joy et al. (2003). That decline was corroborated in the present study, where May was the most important month for larval presence, and September was the least important, as indicated by the chi-square test of equality of proportions, where high chi-square cell contributions for those months accounted for most of the total chi-square value (Table 2). Conversely, monthly prevalences of the 2 Asian species, *Ae. albopictus* and *Oc. japonicus,* and the predatory *Tx. rutilus,* increased throughout the summer. The increase in prevalence for the Asian tiger mosquito was so pronounced that it clearly became the dominant species in September collections. These findings suggest that a competitive pressure is being exerted upon larval populations of the resident *Oc. triseriatus* by invasive species (and possibly by the predatory activities of *Tx. rutilus*) over the breeding season.

Culex restuans also appeared early and exhibited a gradual decline in larval frequency throughout the 2001 breeding season (Joy et al. 2003). This pattern was repeated in 2002 collections, as evidenced by the high proportion of positive collections in May and June, coupled with low positive collections in August and September (Table 2). This pattern is different from that observed by Andreadis (1988), who noted that *Cx. restuans* showed no signs of such a decline, and remained the dominant *Culex* species throughout the summer and fall.

The role of *Cx. territans* as a competitor species is less clear. This species has been reported as an infrequent colonizer of tire habitats (Wilmot et al. 1992, Jamieson et al. 1994, Szumlas et al. 1996), or absent altogether (Lee and Rowley 2000). Conversely, Joy et al. (2003) noted that larvae of *Cx. territans* were the 3rd most commonly encountered forms in West Virginia tire habitats in collections from 2001, although the total positive encounters (~26%) for *Cx. territans* in 2001 was comparable to that (~27%) recorded for 2002 (present study). These prevalences for *Cx. territans* were comparable to the 28% reported for tire dumps in Connecticut (Andreadis 1988).

Lampman et al. (1997) noted that *Cx. territans* was rare in early season collections, and Means (1987) added that larval abundance of this species in New York increased through mid-July and remained stable through late August. Results from both of those studies were corroborated by the present work, as evidenced by the fact that the sum of chi-square cell contributions for the months of May and September made up approximately 83% of the total chi-square value (Table 2).

Ochlerotatus atropalpus was the only species that was evenly distributed across all months of collection, as measured by the chi-square test of equality of proportions (Table 2), and as a result appeared to have no competitive influence on any of the other species. In addition, *Oc. atropalpus* was the only species exhibiting a significantly higher prevalence at nonperidomestic than peridomestic tire larval habitats (Table 1). This is a somewhat different outcome than that observed by Joy et al. (2003), who found that larvae of *Oc. atropalpus* were evenly distributed across both habitat types.

Remaining species were encountered too infrequently to provide valid statistical inferences in terms of their temporal distribution. Still, because of its potential role in the transmission of West Nile virus, *Cx. pipiens* should be considered for continued monitoring. This species is an infrequent colonizer of tires in West Virginia (Joy et al. 2003) and North Carolina (Szumlas et al. 1996), but Wilmot et al. (1992) and Jamieson et al. (1994) noted that *Cx. pipiens* was one of the most common species collected from tires in Wisconsin and Arkansas, respectively, and Andreadis (1988) recorded a prevalence of 55% for this species in tire samples from Connecticut.

In summary, the numbers of larval mosquito species in tires compared favorably with those found in previous investigations. *Ochlerotatus triseriatus* was clearly the dominant larval species in tire habitats, but apparent predation by *Tx. rutilus* and competition by 2 introduced Asian species may contribute to lowered prevalences of *Oc. triseriatus* later in the summer. The desirability for continued monitoring of LAC and WN vectors is discussed because of the potential for increased disease transmission, and the effect(s) of introduced species on resident forms.

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